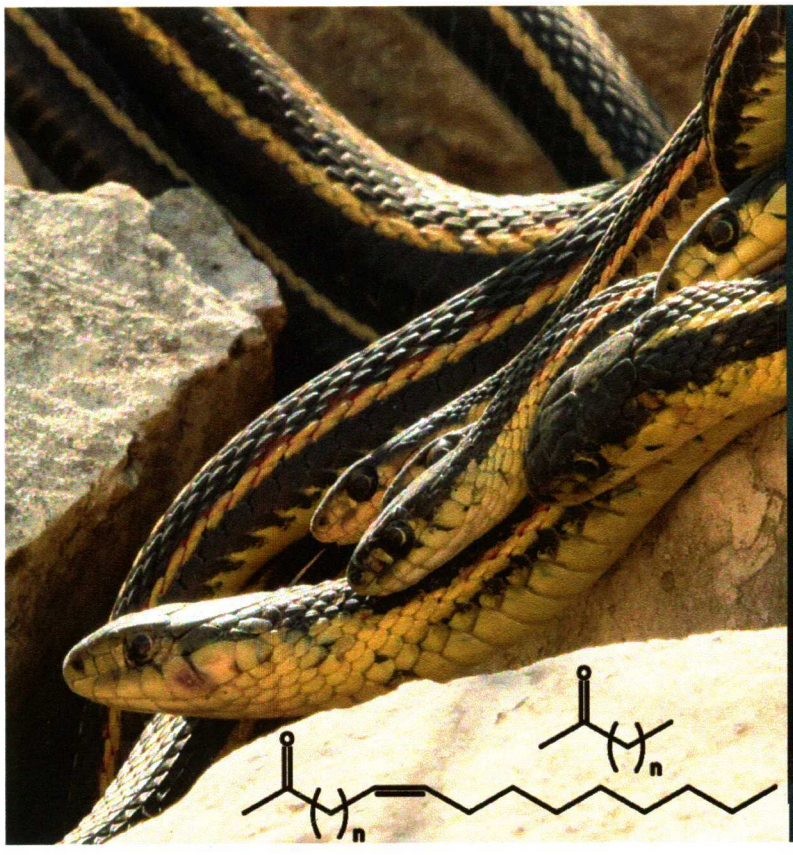
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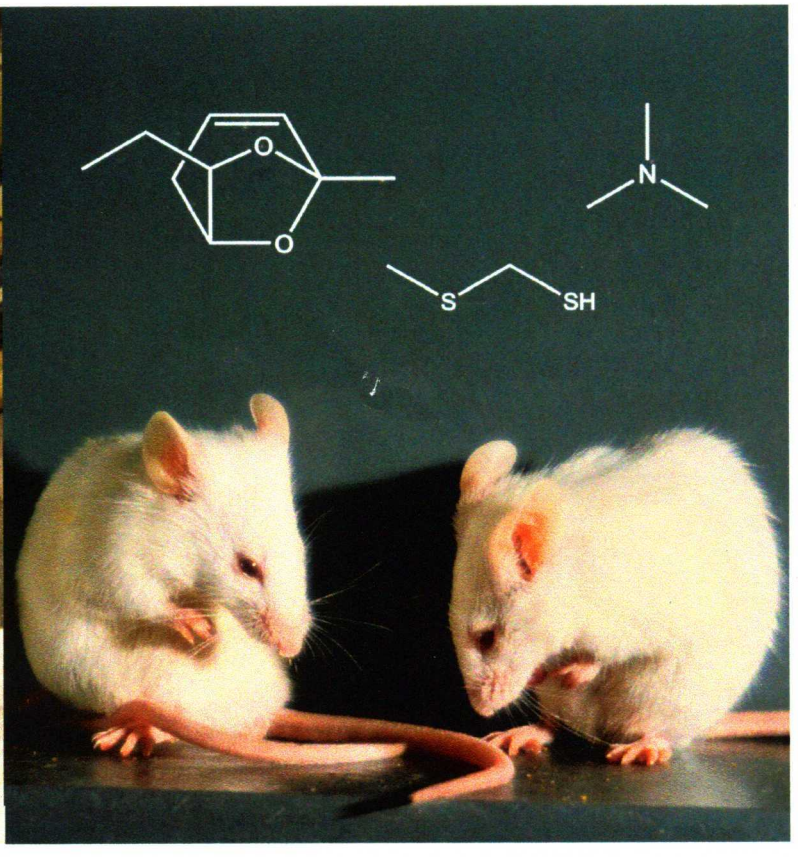
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Chemical signals in terrestrial vertebrates: search for design features

Peter J. Apps,*[a] Paul J. Weldon*[b] and Matthew Kramer[c]

Covering: 1950 to 2015

We compiled a data set of the compounds that terrestrial vertebrates (amniotes) use to send chemical signals, and searched for relationships between signal compound properties and signal function. Overall, relationships were scarce and formed only small-scale patterns. Terrestrial vertebrate signalling compounds are invariably components of complex mixtures of compounds with diverse molecular weights and functionalities. Signal compounds with high molecular weights (MWs) and low vapour pressures, or that are bound to carrier proteins, are detected during direct contact with the source of the signal. Stable compounds with aromatic rings in their structures are more common in signals of social dominance, including territoriality. Aldehydes are emitted from the sender's body rather than from scent marks. Lipocalin pheromones and carriers have a limited range of MWs, possibly to reduce the metabolic costs of their biosynthesis. Design constraints that might channel signal chemistry into patterns have been relaxed by amniote behavior and biochemistry. Amniote olfaction has such a high sensitivity, wide range and narrow resolution that signal detection imposes no practical constraints on the structures of signalling molecules. Diverse metabolic pathways in amniotes and their microbial commensals produce a wide variety of compounds as chemical signals and as matrix compounds that free signal components from the constraints of stability, vapor pressure, species-specificity *etc.* that would otherwise constrain what types of compound operate optimally under different conditions.

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1 Introduction

Chemical signalling is the oldest and the most widespread mode of communication between organisms,[1] and has evolved over more than 3.5 billion years. As a result, the chemicals used to communicate would be expected to exhibit patterned relationships with the messages they transmit, the environmental conditions under which they operate, and the biology of signal

emitters and receivers, because signals must be detectable, discriminable, transmissible, informative, and cost effective. In addition, mechanistic links between signals and the metabolism and physiology of the signaller may be reflected as patterns in signal chemistry. Overall, similar messages sent under similar circumstances would be expected to involve chemicals with similar properties.

The relationship of signal function to the physico-chemical properties of pheromones was first examined by Bossert and Wilson,[2] who focused on the active space, speed of dispersion, and persistence of airborne insect pheromones. Alberts[3] examined chemical signalling by terrestrial vertebrates and found that the chemical composition of signalling odors was related to the type of message and environmental conditions. Mean molecular weight (MW) of odor components differed with the type of message transmitted, and increased in the order of sex attraction, recognition, alarm-threat, and range marking. Range marks contained more compounds with aromatic rings; range marks from hotter and moister habitats had higher mean MWs; and recognition odors and range marks had more components than did sex and alarm-threat odors, possibly because they code individual identity. Alberts[3] concluded that the compositions of mammalian chemical signals are adapted to their function and to the ecology of the communicating organisms.

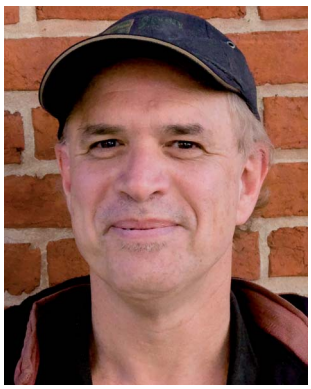
Here, we review current information on intraspecific chemical signals and search for patterns in signal chemistry among modern terrestrial vertebrates (Amniota), including tortoises, squamate reptiles (amphisbaenians, lizards, and snakes), birds, and mammals. The closest living relatives of birds are crocodylians, and birds are now classified as reptiles. Limited data constrained Alberts[3] to assign signalling functions to whole odors and secretions, rather than to specific compounds. More compounds with demonstrated signalling functions subsequently have been characterized, and our search for patterns focuses on these characterized signal compounds.

Our treatment of reptiles complements recent reviews of pheromones[4] and natural products from the integument of nonavian reptiles,[5] and of olfactory signalling[6] and potential semiochemicals in birds.[7] Our treatment of mammals complements reviews emphasizing receiver responses at the neuronal and physiological levels,[8] the role of chemical signals in reproduction,[9] ecological constraints on olfaction,[10] primer pheromones in domestic ungulates,[11] vertebrate pheromones in general,[12] and olfaction across the animal kingdom.[1] Wyatt[13,14] discusses chemical signal design in general terms, and surveys signal design among invertebrates and vertebrates. A special issue of *Hormones and Behavior* on "Chemosignals and Reproduction"[15] contains several up-to-date and detailed reviews.

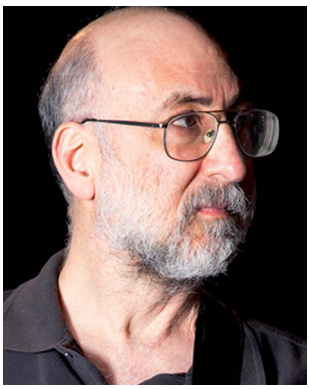
1.1 Olfaction and odor

We use the term "olfaction" in a broad sense to refer to the sensory detection of chemicals that originate outside the detector's body, including dissolved, surface-bound, and airborne compounds, but we exclude gustation. "Odor" refers to substances that are detected by olfaction. Odors are emitted from an animal's body, including its breath, glandular and skin odors, and from scent marks deposited into the environment.

Dr Peter Apps graduated from the University of Oxford with a BA in Zoology in 1977. He received an MSc in Zoology in 1981 and a PhD in Zoology in 1989 for new techniques in the analysis of mammal chemical signals, from the University of Pretoria. He moved from academia to the Onderstepoort Veterinary Institute, from there to the South African Council for Scientific and Industrial Research and then to the National Metrology Institute of South Africa. He has now returned to his roots and analyzes semiochemicals from large African carnivores at the Botswana Predator Conservation Trust's Paul G Allen Family Foundation Laboratory for Wildlife Chemistry.



Dr Paul J. Weldon studied Biology at the Western Connecticut State University. He obtained his Ph.D. in Zoology at the University of Tennessee and was an associate professor in the Department of Biology at Texas A&M University, where he undertook studies of reptiles. After a stay at the National Zoological Park, Washington, D.C., he currently works at the Smithsonian Conservation Biology Institute, Front Royal, VA.



Dr Matthew Kramer graduated from Rutgers University in 1975 with a B.S. in Wildlife Biology, a Ph.D. in Zoology in 1989, and M.S. in Statistics in 1991, both from University of Tennessee. After working at the U.S. Census Bureau until 1999 in time series analysis, he moved to the Agricultural Research Service (USDA), where he is employed in Beltsville, MD as a statistician. He collaborates on a wide range of agriculture-related research projects; pdfs of his articles are available at http://www.ars.usda.gov/pandp/people/people.htm?personid=3122. He also plays jazz guitar at local venues.

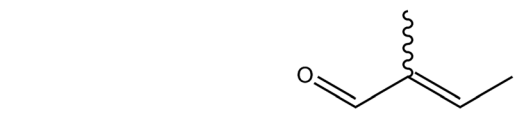
Scent marks include the trails deposited by snakes and other squamates, as well as the discrete urine, fecal, and glandular deposits left by mammals and some lizards.

Terrestrial vertebrates employ three main sensory systems to detect chemicals: the main olfactory system (MOS), the vomeronasal system (VNS), and the gustatory system. The VNS is absent or non-functional in crocodylians, birds, and some mammals. The VNS detects both volatile and non-volatile compounds. No known amniote pheromones are detected by gustation.

1.2 Status as signals and validity of identifications

We restrict the term "chemical signal" to compounds or mixtures whose structures have been elucidated, and that elicit responses at the whole animal level that are similar to those elicited by the natural signal when presented at concentrations similar to those found naturally.[16,17]

Our requirement that a chemical signal has been both bioassayed and characterized excludes hundreds of candidate mammalian signalling compounds for which confirmed structures and bioassays are still required.[18] For example, olfactory communication between mother and young is universal among mammals,[19] but the only characterized semiochemicals that mediate mother-offspring interactions are 2-methylbut-2-enal (**1**) in the milk of European rabbits (*Oryctolagus cuniculus*), which stimulates nipple search;[20] dodecyl propionate (**2**) in the preputial secretion of rat pups (*Rattus norvegicus*), which regulates maternal licking;[21] and corticosterone (**3**) in the milk of rats and mice (*Mus musculus*), which primes the hypothalamic-pituitary-adrenocortical axis of sucklings.[22–24] Among primates, despite detailed work on chemical fingerprints,[25,26] the only signals to have been chemically characterized and bioassayed are three active compounds in thick-tailed bushbabies (*Galago crassicaudatus*), benzyl cyanide, 2-(4-hydroxyphenyl)ethanol, and *p*-hydroxybenzyl cyanide,[27] and the free acid "copulins" of rhesus monkeys (*Macaca mulatta*),[28] whose biological role has been seriously questioned.[29] Males of the >3500 snake species likely use odor to find females, but the only mate-attracting pheromone



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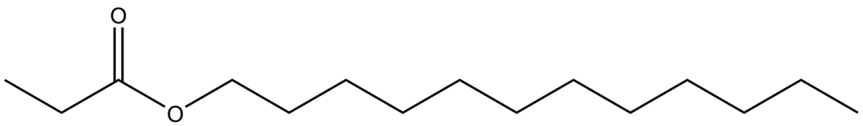

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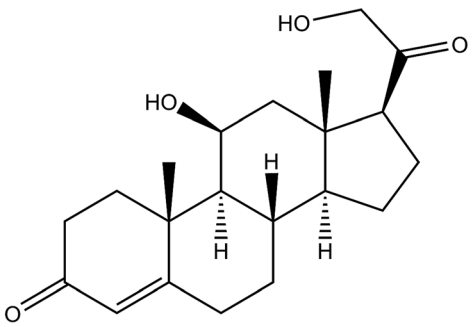

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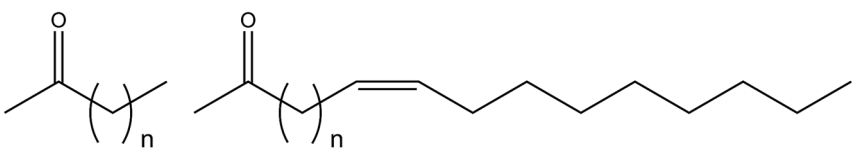

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characterized from snakes are long-chain (C_{29}–C_{37}) saturated (**4–11**) and *Z*-monounsaturated methyl ketones (**12–17**) from female red-sided garter snakes (*Thamnophis sirtalis parietalis*).[30–32]

With the exception of the major urinary protein (MUP) mixtures from male mice, which are unique to individuals and thus constitute signature mixtures according to Wyatt's[33] definition, the signals that we discuss are pheromones according to the original definition by Karlson and Lüscher[34] (p. 55): "… substances which are secreted to the outside by an individual and received by a second individual of the same species, in which they release a specific reaction, for example, a definite behaviour or a developmental process", and to Wyatt's[14] (p. 9) recent operational definition: "fully identified molecule(s), the same across a species, … which when synthesized elicit the same characteristic response in the conspecific receiver as the natural stimulus." The signals discussed here have been chemically characterized by methods that meet criteria for rigorous semiochemical identification.[17,35]

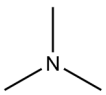
2 Amniote signal chemistry

If the MUPs, whose signalling roles depend on their being a mixture, are counted as one compound, there are 63 characterized compounds with known signalling roles in mammals (Table 1), and 39 in reptiles (Table 2). Some of compounds are parts of multicomponent signals; some have more than one role.

3 Discussion

3.1 Are there patterns, and at what scale?

Considering how few amniote signal compounds have been characterized, they are remarkably diverse. Their MWs range from 59.1 Da (trimethylamine, **18**) to 18 893 Da (darcin), and their structures incorporate 15 functional groups and carbon skeletons with straight and branched chains; various sites of unsaturation and asymmetry; and aliphatic, aromatic, and heterocyclic rings. Different compounds are used to send similar signals, and different signals are sent by similar compounds.



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3.1.1 Exploratory statistical analysis. Statistical analysis can sometimes reveal otherwise obscured patterns. Our questions were (1) are certain chemical categories associated with certain kinds of signals?, and (2) what influences the MWs of pheromones? We confined our statistical analysis to mammals because too few signals are known from reptiles. Even for mammals, limitations of the data set obliged us to take an exploratory approach. The tests are correlated, with the same data used for different analyses, but because we aim to discover new patterns, we did not lower *p* values to control for experiment-wise error. As "positive controls" we included tests of associations that are known to be present *a priori*; for instance, that between signal context and signal type, and between MW and compound class.

3.1.1.1 Variables, classes, and categories. This meta-dataset, like most, suffers from biases inherent when research results are used for analyses not anticipated by the original investigators. Rodents are greatly over-represented, the contexts in which chemical signals are employed are unevenly covered, and the classes of compounds are biased by analytical technique. Our inference space is thus limited, and results are suggestive rather than definitive.

The small numbers of characterized signal compounds, signals, and taxa force us to collapse natural categories into just a few classes for each variable, in order to have sufficient members in each class. We constructed seven classes: chemical class, taxon, signal context, signal type, signal source, carrier, and mode of transmission. Categories of chemical class are "proteins" (including peptides), "hydrocarbons" (including terpenoids), "steroids", "heteroatomic compounds" (nitrogen and sulfur compounds), and "other" (including carboxylic acids, aldehydes, ketones, esters, phenols, and ketals). In addition to being categorized on the basis of overall structure, signal compounds are categorized according to functional group and core structures: steroid nucleus, alcohol, amine, nitrogen, sulfur, nitrogen-heterocycle, sulfur-heterocycle, cyclic, aromatic, lactone, ketone, aldehyde, unsaturated, ester, straight chain, oxygen-heterocycle, hydroxyl, and terpenoid.

Categories of taxa are "rodents", "ungulates", and "other" (lagomorphs, carnivores, and primates). Species also are categorized by diet as "herbivores" or "carnivores and insectivores". Categories of signal context are "dominance/territoriality", "sex," and "other" (which includes mother-offspring interactions). Categories of signal type are "primer", "releaser", and "food mnemonic". Categories of signal effect are "behavioral" and "physiological". Signals in the "behavioral" category include both the classical releaser pheromones and signals with mnemonic effects. The "physiological" category corresponds to the classical "primer" pheromones with developmental and hormonal impacts. Categories of signal source were "body" and "mark". The transmission category entails signal compounds that are airborne and those that are detected by direct contact. The carrier category is divided into "liquid" (urine, tears, and milk) and "solid" (glandular secretions and feces). The signals carried by breath were too few for inclusion.

3.1.1.2 Chi-square (χ^2) analysis. A χ^2 analysis detected significant interactions between biological categories that were expected *a priori* (Table 3). The data for testing interactions of chemical and biological classes are given (Table 4). We found four significant interactions involving signal chemistry; different signal compounds are found in different taxa, in herbivores *versus* carnivores and insectivores, in airborne *versus* contact signals, and in different carriers.

The standardized residuals of each cell in the two-way table for the compound class and taxon interaction show that the three categories of taxa use the compound classes with different frequencies. Although this effect and the association of taxon with signal source likely are artifacts of unequal research effort across signal classes in different taxa, it also may reflect a strong association of diet with taxon, and the possible effects of diet on

Table 1 Characterized signalling compounds from mammals

Compound	Chemical class	MW (Da)	Species and carrier	Effect	References
Trimethylamine	Amine	59.1	Mouse, *Mus musculus*, urine	Species-specific attractant	Li *et al.*, 2013 (ref. 50)
Carbon disulfide	Sulfur	76.1	Mouse breath	Socially transmitted food preferences	Munger *et al.*, 2010 (ref. 87)
(Methylthio)methanethiol	Sulfur	94.2	Male mouse urine	Attracts females	Lin *et al.*, 2005 (ref. 63)
Dehydro-*exo*-brevicomin (7-ethyl-5-methyl-6,8-dioxabicyclo[3.2.1]oct-3-ene)	Ketal	154.2	Male mouse urine	Stimulates male aggression	Novotny *et al.*, 1985, (ref. 193) but see also Chamero *et al.*, 2007 (ref. 167)
2-(*sec*-Butyl)-4,5-dihydrothiazole	Sulfur	143.2			
Dehydro-*exo*-brevicomin	Ketal	154.2	Male mouse urine	Accelerates female puberty[b]	Novotny *et al.*, 1999, 1999 (ref. 166 and 194)
2-(*sec*-Butyl)-4,5-dihydrothiazole	Sulfur	143.2			
6-Hydroxy-6-methyl-3-heptanone	Hydroxy ketone	144.2			
Dehydro-*exo*-brevicomin	Ketal	154.2	Male mouse urine	Attracts females	Ninomiya and Kimura, 1990 (ref. 195)
2-(*sec*-Butyl)-4,5-dihydrothiazole	Sulfur	143.2			
Z-5-Tetradecen-1-ol	Alcohol	212.4	Male mouse urine	Attracts females	Yoshikawa *et al.*, 2013 (ref. 88)
E,*E*-α-farnesene *E*-β-farnesene	Terpenoid	204.4	Dominant male mouse urine	Accelerates female puberty[b]	Ma *et al.*, 1999 (ref. 196)
E,*E*-α-farnesene *E*-β-farnesene	Terpenoid Terpenoid	204.4	Dominant male mouse urine	Aversive to subordinates	Novotny *et al.*, 1990 (ref. 197)
E,*E*-α-farnesene *E*-β-farnesene	Terpenoid	204.4	Dominant male mouse urine	Induces estrus	Ma, Miao *et al.*, 1999; (ref. 196) Novotny *et al.*, 1999 (ref. 166)
2,5-Dimethyl pyrazine	Nitrogen	108.1	Adult female mouse urine	Delays female puberty	Novotny et al., 1986; Jemiolo and Novotny, 1994 (ref. 94)
2-Heptanone	Ketone	114.2			
trans-5-Hepten-2-one	Ketone	112.2			
trans-4-Hepten-2-one	Ketone	112.2			
N-pentyl acetate	Ester	130.2			
cis-2-Penten-1-yl acetate	Ester	128.2			
Corticosterone	Steroid	346.5	Mouse milk	Mother-young developmental primer	Macrì *et al.*, 2007, 2009, 2011 (ref. 24)
Uroguanylin	Peptide	1600.8	Mouse faeces	Transmits food preferences	Arakawa *et al.*, 2013 (ref. 198)
Exocrine gland secreting peptide (ESP) 1	Peptide	7 k	Mouse male tears	Stimulates lordosis in pre-exposed females	Haga *et al.*, 2010; (ref. 177) Kimoto *et al.*, 2007 (ref. 199)
Exocrine gland secreting peptide (ESP) 22	Peptide	10 k	Mouse juvenile tears	Inhibits male sexual activity towards juveniles	Ferrero *et al.*, 2013 (ref. 68)
Major Urinary Proteins (MUPs)	Proteins	18-20 k	Proteins	Sex	Armstrong *et al.*, 2005 (ref. 152)
MUPs	Proteins	18-20 k	Mouse urine	Individual signatures	Hurst *et al.*, 2001 (ref. 220); Kaur *et al.*, 2014 (ref. 84)
MUP3	Protein	18-20 k	Mouse urine	Stimulate male aggression	Chamero *et al.*, 2007 (ref. 167); Kaur *et al.*, 2014 (ref. 84)
Darcin	Protein	18.9 k	Mouse urine	Stimulate male aggression	Kaur *et al.*, 2014 (ref. 84)
Darcin	Protein	18.9 k	Male mouse urine	Female arrestant, mnemonic	Roberts *et al.*, 2010; (ref. 51) Roberts *et al.*, 2012 (ref. 151)
Phenylacetic acid	Aromatic acid	136.1	Male Mongolian gerbil, *Meriones unguiculatus*, ventral gland	Scent mark	Thiessen *et al.*, 1974 (ref. 200)
Aphrodisin	Protein	17 k	Golden hamster, *Mesocricetus auratus*, vaginal discharge	Stimulates copulation in male hamsters	Singer *et al.*, 1986; (ref. 201) Briand *et al.* 2000, 2004 (ref. 130 and 202)

Table 1 (*Contd.*)

Compound	Chemical class	MW (Da)	Species and carrier	Effect	References
Carbon disulfide	Sulfur	76.1	Brown rat, *Rattus norvegicus*, breath	Socially transmitted food preferences	Galef *et al.*, 1988 (ref. 101)
Dodecyl propionate	Ester	242.4	Rat pup preputial gland	Regulates ano-genital licking by females	Brouette-Lahlou *et al.*, 1991 (ref. 21)
Corticosterone	Steroid	346.5	Rat milk	Mother-young developmental primer	Catalani *et al.*, 2000, 2002 (ref. 23)
Squalene	Terpenoid	410.7	Male rat urine	Female attraction	Zhang *et al.*, 2008 (ref. 95); Zhang and Zhang, 2011 (ref. 96)
2-Heptanone	Ketone	114.2			
4-Ethylphenol	Phenol	122.2			
4-Heptanone,	Ketone	114.2			
4-Heptanone,	Sulfur	94.1			
4-Methylphenol	Phenol	108.1			
Propanoic acid	Acid	74.1	Female rat faeces	Signals estrus (erections in male rats)	Nielsen *et al.*, 2011 (ref. 103)
2-Methylpropanoic acid	Acid	88.1			
Butanoic acid	Acid	88.1			
3-Methylbutanoic acid	Acid	102.1			
Pentanoic acid	Acid	102.1			
4-Ethylphenol	Phenol	122.2	Beaver, *Castor canadensis*, castoreum	Site occupation	Müller-Schwarze and Houlihan, 1991 (ref. 97)
1,2-Dihydroxybenzene	Phenol	110.1			
Acetophenone	Ketone	120.1			
3-Hydroxyacetophenone	Ketone	136.1			
Benzoic acid	Aromatic acid	122.1			
5-Methoxysalicylic acid	Phenol	168.1			
4-Hydroxyacetophenone	Hydroxy aromatic	136.1			
2-Methylbut-2-enal	Aldehyde	84.1	European rabbit, *Oryctolagus cuniculus*, milk	Nipple search and attachment, mnemonic trigger for mother's odor	Schaal *et al.*, 2003 (ref. 20)
1,2-Hexadecanediol	Diol	258.4	Sheep, *Ovis aries*, body odor	Ram effect	Cohen-Tannoudji, 1994 (ref. 203)
1,2-Octadecanediol	Diol	286.5			
4-Ethyloctanal[a]	Aldehyde	156.3	Male domestic goat, *Capra hircus*, body odor	Male effect	Murata *et al.*, 2014 (ref. 204)
(*Z*)-4-Hydroxy-6-dodecenoic acid lactone, (enantiomeric ratio 89(*R*)-(−)/11(*S*)-(+)	Fatty acid lactone	196.3	Black-tailed deer, *Odocoileus hemionus columbianus*, tarsal gland and urine	Attractant, stimulates licking	Brownlee *et al.*, 1969; Müller-Schwarze *et al.*, 1976, 1978 (ref. 205)
5α-Androst-16-en-3-ol	Steroid	274.4	Boar, *Sus scrofa*, saliva	Stimulates lordosis	Melrose *et al.*, 1971 (ref. 168)
5α-Androst-16-en-3-one	Steroid	272.4			
4-Methylphenol	Cresol	108.1	Mare, *Equus caballus*, urine	Estrus next day	Būda *et al.*, 2012 (ref. 102)
Cyclohexanone	Ketone	98.1	Asian elephant, *Elephas maximus*, bull temporal gland	Investigation by females (flehmen, trunk scrubbing, palatal pit touches)	Perrin and Rasmussen, 1994 (ref. 206)
Frontalin (1,5-dimethyl-6,8-dioxabicyclo[3.2.1]octane)	Ketal	142.2	Asian elephant bull temporal gland	Musth	Greenwood *et al.*, 2005 (ref. 207)
(*Z*)-7-Dodecen-1-yl acetate	Ester	226.4	Asian elephant female urine	Signals oestrus, stimulates flehmen in bulls	Rasmussen *et al.*, 1997 (ref. 69)

Table 1 (*Contd.*)

Compound	Chemical class	MW (Da)	Species and carrier	Effect	References
4-Heptanone	Ketone	114.2	Red fox, *Vulpes vulpes*, male urine	Stimulates overmarking	Whitten *et al.*, 1980 (ref. 71)
3-Isopentenyl methyl sulfide	Sulfur	116.2			
6-Methyl-5-hepten-2-one	Ketone	126.2			
Benzaldehyde	Aldehyde	106.1			
Acetophenone	Ketone	120.1			
2-Phenylethyl methyl sulfide	Sulfur	152.3			
2-Methylquinoline	Aromatic nitrogen heterocycle	143.2			
Geranylacetone	Ketone	194.3			
2,5-Dimethylpyrazine	Pyrazine	108.1	Male tree shrew, *Tupaia belangeri*, urine	Chinning over-marking	von Stralendorff, 1982, 1987 (ref. 98 and 99)
3-Methylthiopropanoic acid	Sulfur, acid	120.2			
3-Methyl-2-oxovaleric acid	Acid	130.1			
Benzyl cyanide	Nitrogen	117.1	Thick-tailed bushbaby, *Galago crassicaudatus*, chest gland	Over-marking with chest gland	Katsir and Crewe, 1980; (ref. 208) Crewe *et al.*, 1979 (ref. 27)
2-(4-Hydroxyphenyl)ethanol	Alcohol	138.2			
p-Hydroxybenzyl cyanide	Nitrogen	133.1			

[a] Effect probably enhanced by other ethyl-branched carbonyls. [b] Work by Flanagan *et al.*, 2011 (ref. 209) suggests that the Vandenbergh effect (puberty acceleration) is not driven by these compounds alone, and that the complete signal has other unidentified components.

Table 2 Characterized signaling compounds from reptiles, including birds

Compound	Chemical class	MW (Da)	Species and carrier	Effect	References
C_8–C_{18} carboxylic acids	Carboxylic acid	144.2–284.5	Texas tortoise, *Gopherus berlandieri*, chin gland	Shell ramming, aggression	Rose, 1970 (ref. 210)
C_{29}–C_{37} methyl ketones	Ketone	394.7–532.9	Red-sided garter snake, *Thamnophis sirtalis parietalis*, skin	Sex attraction	Mason *et al.*, 1989, 1990; (ref. 30 and 31) LeMaster and Mason, 2002, 2003 (ref. 32)
Squalene	Terpene	410.7	Red-sided garter snake skin	Male recognition	Mason *et al.*, 1989 (ref. 30)
C_{18},C_{24}, C_{26}, and C_{28} alcohols	Alcohol	270.5–410.8	Lizard, *Acanthodactylus boskianus*, femoral gland	Avoidance, aggression	Khannoon *et al.*, 2011 (ref. 211)
Cholesterol	Steroid	386.7			
Squalene	Terpene	410.7	Iberian worm lizard, *Blanus cinereus*, precloacal gland	Biting, aggression	López and Martín, 2009 (ref. 100)
Octanal and *cis*-4-decenal	Aldehyde	100.2 and 182.3	Crested auklet, *Aethia cristatella*, plumage	Attraction	Hagelin *et al.*, 2003; (ref. 181) Douglas, 2008 (ref. 182)
C_6–C_{12} aldehydes					
C_{18}, C_{19}, and C_{20} alcohols	Alcohol	270.5–298.5	Budgerigar, *Melopsittacus undulatus*, uropygial gland	Female attraction to males	Zhang *et al.*, 2010 (ref. 212)
Corticosterone	Steroid	346.5	European starling, *Sturnus vulgaris*, egg yolk	Development	Love *et al.*, 2012 (ref. 22)

Table 3 Significant p values from chi-square analysis of relationships between categories. Empty cells have $p > 0.05$. Relationships in bold are "positive controls" for which high significance is expected *a priori*

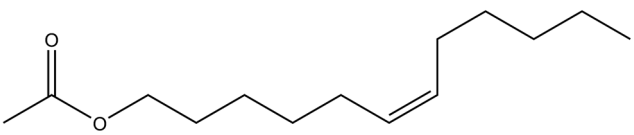
	Signal type	Signal source	Signal effect	Taxon	Diet	Transmission	Carrier	Source	Chemical class
Signal context	**<0.001**	**<0.001**	**<0.001**	<0.001	<0.001	0.043			
Signal type			**<0.001**						
Signal source				<0.001			**0.01**		
Signal effect									
Taxon					**<0.001**	0.049	0.025		0.01
Diet									0.03
Transmission									<0.001
Carrier								0.010	0.045
Source									

substrates for signal biosynthesis (see Section 3.5.2.1). The association of chemical class with mode of transmission is due to steroids and proteins being detected during contact with the signal source (see Section 3.3.1). The marginally significant association of chemical class with carrier probably is an artifact arising from lumping a heterogeneous group of compounds into the "other" category.

3.1.1.3 Analysis of variance (ANOVA) for influences on molecular weight. We examined by ANOVA the relationship between the chemical and biological categorical variables and signal compound MW, log-transformed to stabilize the variance. We started with a model with all main effects and two-way interactions, and used a stepwise procedure to reduce the model using the step function in R.[36] This yielded a model with four main effects and one interaction: compound class ($p < 0.001$), taxon ($p = 0.035$), signal context ($p = 0.008$), signal type ($p < 0.001$), and the compound class by signal context interaction ($p < 0.001$). To understand the relative contribution of each of the independent variables, we did a variance decomposition using the lmer function of the R lme4 package,[37] estimating each component as a random effect. By far the largest contributor to predicting log(MW) is compound class (83.8% of the total variance), because proteins and steroids have high MWs by definition. Since the dominance signal context includes territoriality, and territories often are demarcated by scent marks whose persistence requires signal compounds with low vapor pressures, the significant signal context effect (1.1% of the total variance) was expected (see further discussion in Section 3.3.2). The compound class by signal interaction was driven entirely by one observation, where the prediction of protein pheromone with the lowest log(MW) was adjusted downward (15.0% of the total variance).

3.1.1.4 Discriminant analysis. A standard linear discriminant analysis was conducted to see if the biological variables would separate any of the pheromone classes. This differs from the χ^2 analysis because it shows both which biological variables are the most useful for discriminating among the pheromone classes and which of the classes separate out. We were able to use a slightly less condensed classification of pheromones (although results were almost the same with the coarser classification defined above). The following pheromone classes were used: acid, carbonyl, heteroatom, hydrocarbon, other, protein, and steroid.

The proteins separate from most other compounds on the first discriminant axis (Fig. 1), which is loaded most heavily by transmission (airborne *versus* contact) and carrier. Grouped with the proteins are the two farnesene mixtures that induce estrus and accelerate puberty in female mice, and (*Z*)-7-dodecen-1-yl acetate (**19**), which signals estrus in female Asian elephants (*Elephas maximus*) and which is bound to a carrier protein (see Section 3.3.2). This grouping corresponds with signalling biology and signal chemistry; the grouped compounds have low vapor pressures, are detected during contact with scent marks, and are associated with reproduction. Steroids clump at the lower right, separating from the remainder on the second discriminant axis; two of them signal sex (signal) and two are carried in milk (carrier), both with negative coefficients.



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3.1.1.5 Stepwise discriminant function analysis. The previous discriminant analysis asked whether the pheromone classes could be discriminated. Here we ask if the levels of each variable representing a biological class (*e.g.* carrier, taxon, *etc.*) could be discriminated based on the molecular composition of the pheromones. We used the R klaR package[38] and the lda function of the R MASS package.[39]

A few of the molecular features were useful for discriminating among the biological classes (Table 5). The scarcity of relationships suggests that many of these molecular features have little impact on pheromone use, at least for the categories of biological classes we investigated.

Only a few of the associations between signal chemistry and signal biology have plausible explanations in terms of signal function: amines, proteins, farnesenes, and **19** with detection by direct contact; aromatic rings with dominance signal class; and aldehydes with body sources. For the remainder of the associations, the most parsimonious explanation is that they are artifacts of the unequal coverage of taxa and signals, and the bias inherent to analytical techniques. The influence of the small, biased sample is illustrated by the associations between steroid

Table 4 Counts from cross-classifying chemical with biological classes

		Chemical class				
Biological class	Category	Heteroatomic	Hydrocarbon	Other	Protein	Steroid
Taxon	Other	7	0	8	0	0
	Rodent	9	4	28	9	2
	Ungulate	0	0	8	0	2
Diet	Carnivore	7	0	7	0	0
	Herbivore	9	4	37	9	4
Carrier	Breath	2	0	0	0	2
	Feces	0	0	5	1	0
	Secretion	2	0	16	1	0
	Milk	0	0	1	0	2
	Tears	0	0	0	2	0
	Urine	12	4	22	5	0
Context	Dominance	8	1	17	3	0
	Sex	6	3	24	5	2
	Other	2	0	3	1	2
Effect	Behavior	14	2	34	8	2
	Physiology	2	2	10	1	2
Source	Body	14	4	38	7	4
	Mark	2	0	8	3	2
Transmission	Airborne	16	2	42	0	2
	Contact	0	2	2	9	2
Type	Primer	2	2	10	1	2
	Releaser	12	2	34	7	2
	Mnemonic	2	0	0	1	0

pheromones and signal carrier. The associations are negative with urine because, although steroids are excreted in large quantities in urine, the role of urinary steroids as pheromones has been neglected (see Section 3.5.2.1), and positive with milk and breath because the only known steroid pheromones are carried by breath or milk; this in turn produces a positive association between steroids and signals emitted from the body. Also, primer pheromones have been characterized only from rodents and ungulates, despite evidence that they are widespread among mammals.[22,26,40,41] Such problems are common where results are extracted from the literature.

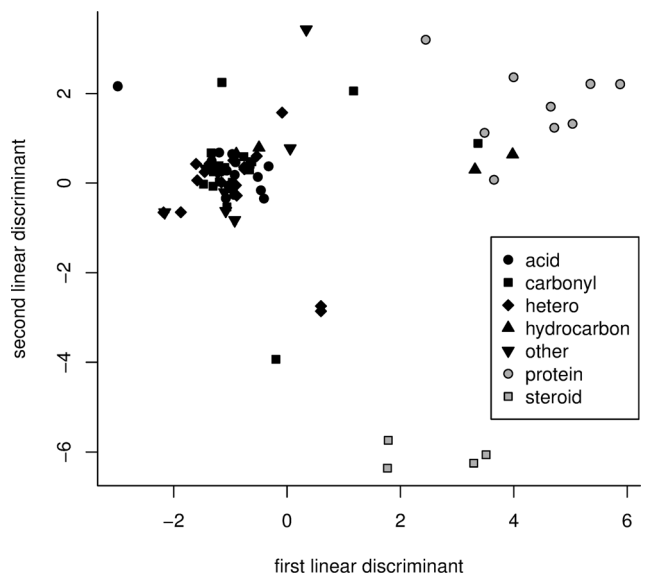


Fig. 1 Compounds (divided into seven classes) as represented in the first and second linear discriminant space. A small amount of noise was added to both axes to better separate the points.

Interactions among some biological categories were expected *a priori*, and their significance in the analyses establishes that where relationships exist they were detected. This, in turn, suggests that if there are any other interactions between signal class and chemical class they would have been detected, deficiencies in the data notwithstanding.

3.1.1.6 Comparison with whole odors. The only statistical analysis similar to ours is by Alberts,[3] who used whole odors rather than signal compounds. As a comparison with her results, we recognized the same chemical classes that she did: carbonyl (aldehyde, ketone, and ester), carboxyl (acid), and hydroxyl (alcohol and phenol). However, due to small sample size in our data set, we included only two of her signal contexts, "sex attractant" (intersexual signals) and "alarm-threat signals (signals observed in aggressive or fear-inducing social interactions)". The data set for this analysis contained 20 carbonyl, 8 carboxyl, and 11 hydroxyl compounds, in 15 alarm-threat and 22 sex (both releaser and primer) signals.

Alberts[3] found that mammalian odor composition was related to signal context, but our analysis of signal compounds failed to indicate a significant interaction between her compound classes and signal contexts ($\chi^2 = 0.1612$, df $= 2$, $p = 0.9225$). Nevertheless, in our stepwise discriminant analysis (see above), aromatic signal compounds most likely were found in the dominance signal class, which includes territorial signals that would have been classified as range marks by Alberts. As Alberts noted, this may be related to the stability of signal compounds (see Section 3.3.2).

Table 5 Molecular groups whose presence/absence was found useful in a stepwise discriminant analysis to separate levels of one or more biological classes. The numbers represent the proportions that the molecular groups were found in that level (*e.g.* a steroid group was found in 50% of compounds in the breath level of the carrier variable). Most molecular groups were not useful for discriminating among levels in any biological class, thus the many blank cells

	Biological class																							
	Carrier					Taxon			Signal class			Effect			Signal type			Source		Transmission		Diet		
Functional group	Breath	Faeces	Milk	Secretion	Tears	Urine	Other	Rodent	Ungulate	Dominance	Other	Sex	Physiology	Behavior	Mnemonic	Releaser	Primer	Body	Mark	Airborne	Contact	Herbivore	Carnivore	
Steroid	0.50	0	0.67	0	0	0												0.29	0					
Alcohol	0	0	0	0.16	0	0.02	0.07	0.02	0.20											0.02	0.60			
Amine																								
Nitrogen																								
Sulfur																								
N-heterocycle																								
S-heterocycle																								
Cyclic																								
Aromatic										0.65	0	0.15												
Lactone																								
Ketone																						0.21	0.36	
Aldehyde							0.13	0	0.10									0.14	0.02					
Unsaturated																								
Ester													0.12	0.02	0	0.02	0.12							
Straight chain																								
O-heterocycle																								
Hydroxy																								
Acid																								
Terpenoid																								

In addition to the patterns that were testable, there are other trends and hints of patterns for which sample sizes are too small. The only feature that generalizes across all characterized amniote chemical signals is that they are coded by a small number of compounds embedded in complex mixtures with diverse chemical characteristics.[18]

Seven peptide or protein signals have been documented for mammals, and none for reptiles. In mammals, five volatile N-containing compounds occur in five signals, and seven volatile S-containing compounds occur in nine signals. Both nitrogen and sulfur occur in seven peptides or proteins that transmit nine signals. Neither N- nor S-containing signal compounds have been found in reptiles.

The non-peptide signals used by mammals have lower MWs than those used by nonavian reptiles, and there are proportionately more multicomponent signals in reptiles than in mammals. Each multicomponent signal in a reptile is a mixture of a single chemical class; either ketones, aldehydes, alcohols or acids, while some multicomponent signals in mammals contain a diversity of compound types.

Ketones are the single most common class of signal compounds in mammals, with 13 compounds identified in five signals, followed by sulfur compounds with seven compounds in nine signals. These numbers of compounds are not significantly different from the occurrence of these compound classes among the components of mammalian odors in general.[42] Strikingly, the rare bicyclic ketals 7-ethyl-5-methyl-6,8-dioxabicyclo[3.2.1]oct-3-ene (dehydro-*exo*-brevicomin, **20**) and 1,5-dimethyl-6,8-dioxabicyclo[3.2.1]octane (frontalin, **21**) signal male breeding status in mice and Asian elephants, respectively (Table 1). Until more species have been examined there is no way to judge whether this is a coincidence.

20 **21**

Overall, there are few links between signal chemistry and biology, and no extensive patterns. Patterns may be obscured by or be artifacts of small sample size and/or uneven coverage of species and types of signals. Only a small fraction of the compounds that are involved in amniote signalling have been characterized, and they are drawn from only 23 (0.09%) of a total of about 23 000 species. The taxonomic coverage is biased by far more work having been done on mammals than on other vertebrates; of the 23 species with characterized signal compounds, 16 are mammals, three are birds, and four are nonavian reptiles.

Within the mammals there is a bias towards laboratory rodents; of the 62 identified mammal pheromone components, 13 are from rats and 22 from mice. Consequently, a large fraction of what we think we know about mammals in general is based on a few inbred strains of two species of small rodents. There is too little detailed work on chemical signalling in other species to assess to what extent laboratory rodents are representative of mammals in general, but there are some indications that they

may not be typical. Laboratory rodents have olfactory receptor genomes that differ from those of other mammals.[43,44] Rats and mice have dozens of intact VNO V2R genes, while dogs (*Canis familiaris*), cows (*Bos taurus*), and primates probably have none. Mice and rats have 187 and 106 functional VNO V1R genes, respectively, while dogs have eight or nine, humans have two (but do not have a VNO), chimpanzees (*Pan troglodytes*) have none, cows have 32, and opossums (*Monodelphis domestica*) have 49.[44–46] Bats, the second most speciose and abundant mammalian order after rodents, do not have VNOs. Between mice and rats, 85–90% of V1R genes are functionally species-specific, but cow V1R genes are 59% and 69% orthologous with sheep (*Ovis aries*) and goat (*Capra hircus*), respectively, and sheep and goat have 97% sequence identity.[47] Mice have 15 trace amine associated receptor (TAAR) genes, dogs have two, opossums have 21, and platypuses (*Ornithorhynchus anatinus*) have four. With three TAARs, chickens (*Gallus gallus domesticus*) are more similar to dogs than dogs are to mice or opossums.[44,46] Mice may be outliers in terms of signal composition; MUP type proteins are widespread in rodents, but in most species do not show the diversity that makes them into signature mixtures in *M. musculus domesticus*.[48] We have a very detailed map of one small corner of amniote semiochemistry, and a few landmarks are visible in the distance, but the landscape in between is *terra incognita*, and this very uneven coverage is expected to make patterns difficult to discern.

Patterns in signal chemistry may be obscured by limitations in chemical analyses. In terrestrial vertebrate semiochemistry, the most commonly applied analytical technique is gas chromatography-mass spectrometry (GC-MS), usually with methods that exclude compounds with MWs above about 400 Da or that need derivatization. Analyses of peptide and protein semiochemicals have been applied to far fewer species than GC-MS, and have characterized chemical signals only in mice and golden hamsters (*Mesocricetus auratus*).

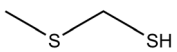
It is tempting to dismiss the scarcity of discernible patterns among amniote chemical signals as an artifact of small, biased samples and analytical technique. Alternatively, the scarcity of patterns may accurately represent the chemical diversity of amniote chemical signals because, as we argue below, the properties of terrestrial vertebrate olfactory systems and the chemistry of their odors frees signalling compounds from many of the constraints that might otherwise generate patterns in signal chemistry.

3.2 Signal detectability

3.2.1 Scope and sensitivity of amniote olfactory detection. The amniote olfactory system has a remarkably wide scope. The MWs of molecules that amniotes are known to detect range from 17 Da for ammonia to 7.4 kDa for crotatoxins, which are detected by rattlesnakes (*Crotalus* spp.).[49] The smallest signal molecule is **18** (59.1 Da)[50] and the largest is darcin (1.8893 kDa).[51] The huge diversity of molecules detectable by mammals includes every known naturally occurring organic functional group.

Contrary to presumptions that only the VNO detects pheromones, and that only pheromones are detected by the VNO,[52] signal compounds are not constrained to be detectable by the VNO. In both reptiles and mammals the VNO detects molecules with no known signalling function,[53,54] and in mammals the MOS detects pheromones.[55] The few pheromones that have been identified from squamates appear to be detected by the VNO, but, long, up-wind excursions by male rattlesnakes to females indicate their use of airborne pheromones possibly detected by the MOS.[56] The copulatory fluids of male red-sided garter snakes emit airborne pheromones that terminate courtship in prospective rivals.[57]

Chemical signals might be expected to be compounds whose receptors exhibit particularly low limits of detection (LODs). This expectation is not borne out; receptors with unusually low LODs (Table 6) are scattered throughout olfactory space and are not confined to detectors of chemical signals. For example, the lowest measured olfactory LOD in domestic dogs is 5×10^{-14} mol.mol^{-1} for α-ionone,[58] and in mice it is 10^{-16} mol.mol^{-1} for bourgeonal (3-(4-*tert*-butylphenyl)propanal),[59] but neither compound is a chemical signal. Male golden hamsters respond to 500 fg of dimethyl disulfide,[60] which is a general attractant but not a hamster pheromone.[61] Compared to the LODs for general odors the olfactory LODs for some known pheromones are not particularly low: sows (*Sus scrofa*) detect the pheromone 5α-androst-16-en-3-one (**22**) at 3×10^{-4} M and geraniol (a terpene alcohol with a floral odor) at 3.5×10^{-7} M.[62] Female mice respond to the male pheromone (methylthio)methanethiol (**23**) spiked into castrate urine at 0.2 μM (ref. 63) and to aliphatic acids, which are not pheromones, at 1 nmol mol^{-1} to 3 pmol mol^{-1} in the gas phase.[64]



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Comparisons between whole animal and neurophysiological LODs are complicated by the fact that animals have been tested with airborne odors whose concentrations are expressed as mole fractions, or as mass fractions of the solution with which they are equilibrated, while physiological preparations have been tested by directly applying solutions, with concentrations usually expressed in molarity. With that limitation, LODs are similar for general odors and signal compounds (Tables 6 and 7), and low LODs do not necessarily point to a signalling role. For example, the TAAR5 receptor in rats has a LOD of 150 nM for **18**, which is not a rat pheromone.[50] Sulfur compounds with no known semiochemical activity are detected by the mouse olfactory receptor MOR244-3 at 10 nM, which is similar to its LOD for the female-attracting pheromone **23**.[65,66]

Compared to the natural concentrations of signal compounds, mammalian olfaction has sensitivity to spare. The mouse VNO responds to urine diluted by a factor of 100 000, and its most sensitive neurons respond to dilutions of 100 000 000.[67] The LOD of TAAR5 for **18** is 100 000 times lower than the concentration of **18** in male mouse urine,[50] and the LOD for ESP22 is 20 000 times lower than its concentration in the tears of juvenile mice.[68] The concentration of **19** in the urine of female Asian elephants reaches 0.146 mM just before ovulation,[69] steroid pheromones in boar (*Sus scrofa*) saliva occur at low μg ml^{-1},[70] and active constituents in the urine of male red foxes (*Vulpes vulpes*) occur at μg ml^{-1}.[71] All these concentrations

Table 6 Selected detection thresholds for whole animal olfaction. Where ranges were reported, the threshold shown by the most subjects is given

Compound	Species	Threshold (matrix)	Ref.
2,4,5-Trimethylthiazoline (fox odor)	Rat, *Rattus norvegicus*	1.1 $pg.g^{-1}$ (solvent)	Laska *et al.*, 2005 (ref. 213)
2,4,5-Trimethylthiazoline	Pigtail macaque, *Macaca nemestrina*	4 $ng.g^{-1}$ (solvent)	Laska *et al.*, 2005 (ref. 213)
2,4,5-Trimethylthiazoline	Squirrel monkey, *Saimiri sciureus*	14 $ng.g^{-1}$ (solvent)	Laska *et al.*, 2005 (ref. 213)
2,4,5-Trimethylthiazoline	Spider monkey, *Ateles geoffroyi*	1.4 $ng.g^{-1}$ (solvent)	Laska *et al.*, 2005 (ref. 213)
Ethanethiol	Spider monkey	10 $pmol.mol^{-1}$ (air)	Laska *et al.*, 2007 (ref. 214)
3-Methyl indole	Spider monkey	10 $pmol.mol^{-1}$ (air)	Laska *et al.*, 2007 (ref. 214)
Dimethyl sulfide	Harbor seal, *Phoca vitulina*	450 $amol.mol^{-1}$ (air)	Kowalewsky *et al.*, 2006 (ref. 215)
n-Hexanal	Mouse, *Mus musculus*	30 pM (solvent)	Løtvedt *et al.*, 2012 (ref. 216)
cis-3-Hexenal	Mouse	1.1 nM (solvent)	Løtvedt *et al.*, 2012 (ref. 216)
Bourgeonal (3-(4-*tert*-butylphenyl)propanal)	Mouse	100 $amol.mol^{-1}$ (air)	Larsson and Laska, 2011 (ref. 59)
α-Ionone	Dog, *Canis familiaris*	50 $fmol.mol^{-1}$ (air)	Moulton and Marshall, 1976 (ref. 58)

Table 7 Detection thresholds of vomeronasal organ (VNO) and main olfactory epithelial (MOE) sensory neurons for semiochemicals

Compound	Species and olfactory subsystem	Detection threshold (M) in buffer	References
2,5-Dimethylpyrazine	Mouse VNO	10^{-8} to 10^{-7}	Leinders-Zufall *et al.*, 2000 (ref. 217)
2-*sec*-Butyl-4,5-dihydrothiazole	Mouse VNO	10^{-10} to 10^{-9}	Leinders-Zufall *et al.*, 2000 (ref. 217)
2,3-Dehydro-*exo*-brevicomin	Mouse VNO	10^{-10} to 10^{-9}	Leinders-Zufall *et al.*, 2000 (ref. 217)
Mixture of *E*,*E*-α-farnesene and *E*-β-farnesene	Mouse VNO	10^{-11} to 10^{-10}	Leinders-Zufall *et al.*, 2000 (ref. 217)
2-Heptanone	Mouse VNO	10^{-11} to 10^{-10}	Leinders-Zufall *et al.*, 2000 (ref. 217)
2-Heptanone	Mouse VNO V1rb2	10^{-10}	Boschat *et al.*, 2002; (ref. 218) Spehr *et al.*, 2006 (ref. 78)
6-Hydroxy-6-methyl-3-heptanone	Mouse VNO	10^{-8} to 10^{-7}	Leinders-Zufall *et al.*, 2000 (ref. 217)
ESP1	Mouse V2Rp5 in VNO	10^{-7}	Haga *et al.*, 2010 (ref. 177)
ESP22	Mouse VNO	2×10^{-11}	Ferrero *et al.*, 2013 (ref. 68)
MHC peptides	Mouse VNO	10^{-11} to 10^{-12}	Leinders-Zufall *et al.*, 2004 (ref. 83)
Individual MHC peptides	Mouse VNO	10^{-12}	Overath *et al.*, 2014 (ref. 162)
(Methylthio)methanethiol	Mouse MOR244-3 in MOE	10^{-8}	Block and Zhuang, 2013 (ref. 66)
Carbon disulfide	Mouse MOE	1.3×10^{-7}	Munger *et al.*, 2010 (ref. 219)
Trimethylamine	Mouse TAAR5 in MOE	10^{-8}	Li *et al.*, 2013 (ref. 50)
MHC peptides	Mouse MOE	10^{-11}	Spehr *et al.*, 2006 (ref. 78)

are at least three orders of magnitude above the LODs for mammalian olfaction. In sharp contrast, the quantity of pheromone on one female garter snake is only just above the LOD of male garter snakes.[31] Clearly, signal compounds are not constrained to have chemical structures that allow them to be detected by sensory neurons with unusually low LODs.

3.2.2 Access to sensory epithelia. An obvious constraint on detectability is imposed by the need for signal compounds to reach chemosensory epithelia. The sensory epithelium of the MOS, the main olfactory epithelium (MOE), is exposed to inhaled and exhaled air, and is most accessible to airborne substances. The VNO sensory epithelium is on the roof of the mouth in most reptiles, and in a liquid-filled diverticulum of the nasal or oral cavities in mammals. Molecules are transferred to it by tongue flicking in squamate reptiles, by flehmen in ungulates and carnivores, and by a vascular pump in rodents.[72–74] Patterns in signal chemistry might be generated by constraints on what types of compounds can reach the sensory epithelia, for instance, if only hydrophilic molecules can reach the VNO, and only volatiles can reach the MOE. Adaptations to circumvent these chemical limitations occur in snakes and mammals. In red-sided garter snakes, the lumen of the VNO is filled with liquid that solubilizes the hydrophobic long-chain methyl ketones of the male attracting pheromone.[75] In Asian elephant bulls, the trunk mucus contains an 18.5 kDa odorant-binding protein that binds the hydrophobic estrus pheromone **19** from female urine. The trunk transfers **19** in the free form to the MOE and as the protein-**19** complex to the VNO. In the VNO ducts, **19** transfers from the odorant-binding protein to a 60 kDa albumin that shuttles it to the sensory neurons.[76] Like **19**, the rat pup preputial pheromone **2** is very hydrophobic and is detected by the VNO,[77] but whether a carrier protein is involved has not been established. Vigorous sniffing and licking of female urine by male mice transfers non-volatile major histocompatability complex peptides to their MOEs.[78] Thus, although there is a trend for non-volatile signals to be detected by the VNO, and more volatile ones by the MOE, signal chemistry is not narrowly constrained by access to the sensory epithelia.

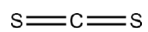
3.2.3 Selectivity of olfaction. The olfactory system's responses to chemical stimuli, including chemical signals, are "selective" and "specific" in the same sense that these terms are used in analytical chemistry.[79] A specific response occurs only to

one particular substance, while a selective response occurs to two or more substances, which usually have some property in common. Specificity is the highest level of selectivity.

Chemical communication requires chemical signals to stand out from the "chemical cacophony"[13] of general chemical background, the "olfactory cocktail party"[80] of odors from other animals, and the other constituents of the odor in which it is embedded. Chemical differences between signal compounds and potential interferences must be large enough for the olfactory system to respond selectively to the signal compounds. The more selective the olfactory system is, the smaller the structural differences between compounds that can be discriminated, and the larger the number of compounds that can be used as signals. If olfaction is not selective, each compound in the chemical background is surrounded in chemical space by a large number of structurally similar compounds that cannot be used as signals, and signals will be absent from parts of chemical space that are occupied by background compounds. Such gaps in signal space could give rise to recognizable patterns in signal chemistry.

Amniote olfaction is exceptionally selective, as well as having a very wide scope. Even humans, with no functional VNO and only 350 intact olfactory receptor (OR) genes, are estimated to discriminate the odors of over one trillion chemical mixtures.[81] Mammals routinely make fine olfactory discriminations between complex mixtures that have no connection with signalling,[80,82] showing that the mammalian olfactory system has more selectivity and scope than are required for signal discrimination. Consequently, signal chemistry is not significantly constrained by discriminability, and the selectivity of olfactory detection, combined with its wide scope, is too high to have generated recognizable patterns in signal chemistry.

Sex-specific responses to female sex pheromones in red-sided garter snakes are generated at the receptor level; only males have VNO receptors that respond to the relevant methyl ketones.[75] Specificity and selectivity in the mammalian VNO also reside mainly at the receptor level, mediated by a mixture of specific and broadly selective detectors.[83,84] In the mouse VNO, there are specific detectors for the MUP3 pheromone,[84] and sensory neuron V2Rp5 is specific for the peptide pheromone ESP1.[85] Selectivity in the MOS is generated mainly by combinatorial coding of signals from diverse broadly-tuned receptors;[86] each olfactory sensory neuron in the MOE expresses one olfactory receptor protein that is broadly selective for molecules within a particular range of structures. The MOE also has receptors that are specific for amines,[50] and the breath-borne pheromone carbon disulfide (**24**) has a dedicated olfactory subsystem in mice.[87]



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It is important to note that broadly-tuned receptors can be specific for single components of socially relevant odor mixtures. For instance, the mouse olfactory receptor MOR244-3 is broadly selective for a suite of sulfur compounds,[65,66] but only one of these, the male pheromone **23**,[63] occurs in mouse urine and, under natural conditions the broad selectivity generates a pheromone-specific response. Although the main olfactory receptors (MORs) of mice are broadly selective when exposed to general odors, 80% of them are specific to single compounds among the subset of volatiles that are emitted by mouse urine.[63] MOR Olfr288 responds to several odorants, but the only one of them that occurs in mouse urine is (*Z*)-5-tetradecen-1-ol, which attracts females.[88] This suggests that the structural differences between signal compounds and the other components of the odors in which they are embedded are a potential source of patterns in signal chemistry that is worth investigating.

3.2.3.1 Species-specificity. In the wild, in marked contrast to the single-species systems of laboratory and domestic animals where semiochemical research is focused, most vertebrate species share their habitats with thousands of individuals from dozens of other vertebrate species. All of these individuals emit odors and leave scent marks, but only those from an individual's own species are socially or reproductively relevant. Specific mate-recognition signals must self-evidently be species-specific,[89] and so also must most social signals. Odors from other populations are a major source of potential interference with chemical signals, and on this basis, chemical signals would be expected to be unique to a species or a population. This is especially true for scent marks, which must operate in the absence of the animals that deposit them.[90]

All amniote chemical signals are embedded in complex mixtures of other odors. No two species have been found to emit the same mixture of compounds, and there are numerous examples of interspecific differences that have not yet been shown to be signals of species identity (reviewed in Apps[18]). Other compounds have established roles as species-specific signals. **18** is a species-specific attractant for mice, and male urine from *Mus musculus musculus* (strain C57BL/6) contains about 1000 times as much **18** as does the urine of male rats, and approximately 300 times as much as urine from male *M. spretus*, *M. m. domesticus* and *M. spicilegus*.[50] *Mus musculus domesticus*, *M. m. musculus*, and *M. m. castaneus* have three different alleles (*Abpa*^{a} in *domesticus*, *Abpa*^{b} in *musculus*, and *Abpa*^{c} in *castaneus*) of the gene that codes for androgen-binding protein in saliva.[91] Males mark their territories with the protein and its influence on female mate choice may maintain reproductive isolation among the three subspecies (Laukaitis *et al.* 1997).[92] The long-chain ketone pheromone profiles of garter snakes (*Thamnophis* spp.) differ more between sympatric than allopatric species (Uhrig *et al.* 2014),[93] presumably a reflection of reproductive isolation maintained among overlapping congeneric populations.

Despite the expectation of species-specific signal chemistry, several signal components are not species-specific (Tables 1 and 2). 2-Heptanone is part of a multicomponent signal in female mouse urine that delays puberty in immature mice,[94] and part of a multicomponent signal in male rat urine that attracts female rats.[95,96] Another component of the male rat urine signal, 4-ethylphenol, also occurs in beaver (*Castor canadensis*) castoreum as part of a multicomponent signal of range occupation.[97] Both castoreum and fox urine contain acetophenone as a component of multicomponent signals that stimulates overmarking.[71,97] 2,5-Dimethylpyrazine is part of the multicomponent puberty-delaying signal in the urine of female mice[94] and is one of three volatiles in the urine of male tree shrews (*Tupaia belangeri*) that stimulate

overmarking.[98,99] Squalene and cholesterol are widespread in vertebrate secretions. Squalene is part of a female-attracting mixture in the urine of male rats[95,96] and part of a male signal in the red-sided garter snake;[29] both compounds elicit aggression in squamates.[100] **24** facilitates social transmission of food preferences in both rats and mice.[87,101] **3** primes developmental trajectories in mice, rats, and European starlings (*Sturnus vulgaris*).[22–24] 4-Methylphenol is an almost ubiquitous component of mammalian urines, and acts as a signal of estrus in mares (*Equus caballus*)[102] and a female attractant in male rat urine.[96] A mixture of five short-chain aliphatic acids whose concentration in feces declines when female rats, horses (*Equus ferus caballus*), or red foxes are in estrus, shows an inverted-U-shaped dose–response curve in eliciting erections in male rats when spiked into female rat feces.[103] The male effect in sheep (*Ovis aries aries*) and goats (*Capra aegagrus hircus*), in which estrus in ewes or nannies is stimulated by luteinizing hormone (LH) pulses that are triggered by a male's body odor, is not species-specific; billy goat odor triggers LH pulses in ewes[104] and ram odor triggers gonadotropin releasing hormone/LH release in nanny goats.[105]

3 in milk and **24** in breath do not need to be species-specific because they are transferred direct from one individual to another and there is no possibility of inter-specific cross talk. The estrus signal in the urine of mares does not need to be species-specific because mares urinate in response to investigation by stallions, and their species identity is mutually available from other cues. If two species do not co-occur, as with the ancestors of sheep and goats, they will not receive one another's signals, and species-specificity is then irrelevant. It is when signal compounds are deposited as components of scent marks, and emitted in the absence of the marker, that additional features of each species' signals are needed to inform conspecifics that the signal is relevant. Correspondingly, there is a pattern in signal design; all signals that are emitted from scent marks and that contain a compound emitted by another species are multicomponent signals, and with the exception of the acid mixture that signals estrus, at least one of the components in each of them is species-specific. Nevertheless, because a difference in only a single compound is sufficient to chemically differentiate two species,[106] species-specificity imposes no practical constraint on signal chemistry.

Even when odors are not species-specific, species-specific responses can be generated at the level of the olfactory receptors or by processing at higher levels in the central nervous system. Grus and Zhang[46] found that VNO receptor genes differed between mouse, rat, dog, opossum, and platypus. There are also large differences in main olfactory receptor (MOR) genes between species. The number of known receptor proteins varies from 296 in orangutans (*Pongo pygmaeus abelii*) to 1948 in African elephants (*Loxodonta africana*).[107] Among mice, dogs, and humans, out of 412 MOR gene subfamilies, 34 subfamilies occur only in dogs, 60 only in humans, and 69 only in mice,[108] but mice have only four intact MOR genes that are not found in rats, and rats have only one intact MOR gene that is not found in mice.[109] V2R VNO receptors in *Mus musculus* are specific for the odors of species and subspecies of *Mus*.[110]

Species-specificity can reside at levels above the receptors. **18** attracts female mice and repels rats and many other species.[49] Since all of the species can smell it, the aversive and attractive effects of the same chemical in different species must be due to different processing within the central nervous system. In mice and rats, species-specific processing of sensory input from the VNO occurs in the amygdala.[111]

Species-specific responses provide a straightforward mechanism for species-specific signalling when different species have most of their odor components in common. In principle, it would be possible for different species to read species-specific messages from different components of the same mixture of compounds. If species-specificity is unnecessary, or is not conferred by chemical composition, then a further constraint on the chemical composition of signals is removed, and there are less likely to be patterns in signal chemistry.

3.3 Transmission

3.3.1 Active space and detection at a distance. Most scent marks are visually inconspicuous, and if they are detected from a distance it must be through airborne odors. Hard data on the distances over which scent marks can be detected, or how tetrapods find scent marks, are scarce. Mice can detect fresh urine from at least 15 cm away,[112] but indirect evidence from the detection of target odors by search dogs does not support robust scent-mark detection distances of much more than *ca.* 10 m in large mammals.[113] Odor transport at the spatial scale relevant to terrestrial vertebrates depends on advection, not diffusion; signals are blown downwind and dispersed by turbulence.[14] For volatiles, mass transport by advection is at least three orders of magnitude faster than by diffusion[2] and is independent of MW, which raises the upper limit of MWs that can generate realistic active signal volumes. The limit would be raised still further if molecules that are too heavy to be volatile are adsorbed onto airborne particles and aerosols, which move with air currents but do not diffuse.[114] As a dog sniffs at a scent source, the air flow around its nostrils disturbs particles that are inhaled,[115] mouse MUPs can be airborne,[116] and synthetic boar pheromones to detect estrus in sows are commercially available in aerosol cans, but there have been no systematic studies on whether airborne signal chemicals are transported on particles. There is nothing to suggest that signal compounds in scent marks have special properties that allow long-range transmission.

Because material can be transferred in bulk to both the VNO and the MOE, there are no obvious constraints on MW or functionality for compounds that are detected during physical contact with either the signalling individual or a scent mark. As expected, proteins, farnesene, and **19** which have low vapor pressures are detected during contact (Fig. 1 and Table 5), but so is the compound with the second lowest MW and the highest vapor pressure among mammalian pheromones: 76.1 Da **24** in mouse and rat breath.[87,101] Direct contact detection is widespread; squamates sample surfaces by tongue flicking, in ungulates, males and some females nuzzle and lick a female's genitals, sample the urine that she produces in response, and pump it to the VNO using the flehmen grimace.[73] Cats (Felidae), especially males, use flehmen to

sample fresh urine.[72] Nocturnal prosimians sniff, lick, flehmen, bite, and even swallow urine scent marks.[117] Corticosteroids in milk are ingested directly by sucklings.[22,23,40]

3.3.2 Persistence. Because body odors can be produced and emitted continuously, any compound that survives even briefly in light and air could serve as a signal. In contrast, scent marks need to be persistent. Aldehydes, which are susceptible to oxidation, are less common in scent marks and more common in body odors (Table 1). The stability of aromatic compounds may account for their being more common in range marks[3] and in dominance signals, which include territorial scent marks (Table 1). Heavier molecules with low vapor pressures will be emitted more slowly, and for a given quantity of compound in a scent mark, their emissions will persist for longer than those of smaller molecules. Nevertheless, their slow emission produces low gas phase concentrations, and if marks are detected *via* airborne components, this will render them less detectable. These competing requirements for persistence and detectability could produce a pattern in signal chemistry by limiting signal compounds to a narrow range of MWs and functional groups.

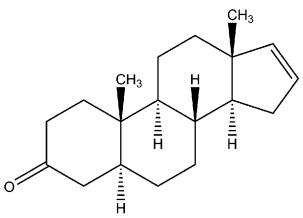
Many squamates continually sample substrate-borne chemicals by tongue flicking, and so their signal detection does not depend on compounds in the gas phase. The chemical signals in squamate scent marks can be remarkably persistent; prairie rattlesnakes (*Crotalus viridis*) locate overwintering dens by following trails left by conspecifics months beforehand.[118] The chemical signals in squamate scent marks have higher MWs and lower vapor pressures than do the non-peptide signal compounds in mammalian scent marks (Tables 1 and 2).

Not all scent marks emit signals over very long periods. Interest by male hamsters in the flank and vaginal marks of females declines sharply after 30 min.[119] Female mouse urine elicits unconditioned ultrasonic squeaks from males for 15–18 h.[120] The repellent effect of dominant male mouse urine on other males persists for between 1 h and 24 h,[121] but mice respond to the MUPs in the urine for at least seven days and MUPs can be detected analytically weeks after being deposited.[51,112] Klipspringer (*Oreotragus oreotragus*) marks last for seven days,[122] and follicular phase urine voided by Asian elephant cows attracts maximal interest from bulls for 1–2 days.[76]

Although the loss of signal compounds from scent marks is often ascribed to evaporation, it may not be the only mechanism of signal fade-out. Semiochemically active **20** is converted to *exo*-brevicomin as mouse urine dries and ages,[123] and the disappearance of an ephemeral signal chemical from female mouse urine does not occur in the presence of anti-oxidants.[124] These various mechanisms of signal fade-out are an additional source of the chemical diversity that is likely to disrupt patterns in signal chemistry.

Some scent marks emit odors (but not necessarily signals) for much longer than can be accounted for by simple evaporation. Dwarf mongooses (*Helogale parvula*) can detect 20–25 day-old anal gland secretion.[125] Male guinea pigs (*Cavia porcellus*) discriminate the sex of the donors of dry urine films that are 40 days old.[126] Interest by male hamsters persists for 40 days for male flank marks and 100 days for vaginal marks.[119] Male Coquerel's mouse-lemurs (*Microcebus coquereli*) discriminate urine films dried on glass after six months, and rehydrated dried films after 20 months.[117] Unless the long-lived emissions are of compounds with very high MWs, these lifetimes are almost certainly due to fixatives that retard the emission of low MW compounds.[127]

A role as fixatives probably accounts for the presence in Asian elephant temporal gland secretion of high MW proteins, lipids, and steroids with no apparent signal activity,[128] and the presence in saddle-back tamarin (*Saguinus fuscicollis*) scent marks of high MW esters and squalene that make up 96% of the mark but which tamarins do not discriminate from odorless controls.[129] Aphrodisin in the urine of female golden hamsters binds the attractant dimethyl disulfide.[130] Seventeen mouse urine volatiles are bound to MUPs, and individually specific odor signatures are generated by interactions between volatiles and different MUP mixtures.[131] As a result of the volatiles–MUP interactions, one set of compounds (the volatiles) that dissipates too quickly to produce persistent marks with stable signatures combines with another set of compounds (the MUPs) that have no active space to produce scent marks that are attractive, stable, persistent, and specific to their depositor. The boar pheromones, **22** and 5α-androst-16-en-3-ol (**25**), are concentrated into saliva by binding to pheromaxein, a 15 kDa secretoglobin protein.[132] The two pheromones are released from the protein by its microbial degradation.[133]



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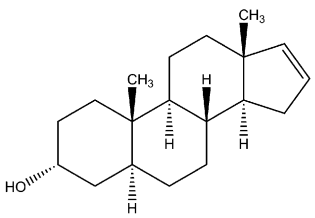

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There are two clear examples of a scent mark matrix being manipulated to change a chemical signal. In European rabbits, dominant males increase the lifetimes of their chin gland secretions by secreting more of the fixative 2-phenoxyethanol, which rabbits cannot smell.[134] Male ring-tailed lemurs (*Lemur catta*) mix volatile brachial gland secretion with heavier antebrachial gland secretion, which acts as a fixative, before depositing the mixture as scent marks.[26]

Although its possible role as a fixative has not been recognized, urea, which occurs in all mammalian urine, can form inclusion complexes with unbranched carbon chains bearing a variety of terminal functional groups. Complexation is remarkably selective and is reversible at temperatures around 25 °C.[135] Urea complexes form only in the solid phase, and this may explain why liquid female guinea pig urine loses its attractiveness to males after 48 h,[136] but as a dry film it is attractive and discriminable from male urine after 40 days.[126] In addition to urea forming inclusion complexes with volatiles, odor-binding proteins might be denatured by increasing urea concentrations as urine dries, giving rise to serial emission of the volatile ligands as their protein carriers are sequentially denatured in drying

urine films. The equilibrium concentrations of volatiles above mouse urine change with time due to drying and slow release from MUPs,[123,137] and in this context it is interesting that darcin is stable to 7.5 M urea, while MUP 11 is denatured at 6 M.[138]

If the signal compound emissions of a scent mark remain latent until it is encountered and manipulated by a receiver, its lifetime may become largely independent of environmental conditions, and be better measured by how often it can be "read" than by time.[3] The release of volatiles from scent marks in response to sniffing and licking is most likely due simply to the addition of moisture,[127] but there are also specific displacers in saliva, nasal mucus or breath that liberate signals from their carriers. The saliva of Mongolian gerbils (*Meriones unguiculatus*) specifically liberates components from their harderian gland secretions.[139] The Asian elephant estrus pheromone **19**, which is hydrophobic, is selectively bound by a 66 kDa albumin in urine. The binding depends on pH; each albumin molecule binds four molecules of **19** at pH 8.4, and none at pH 5. When a bull samples a female's urine by placing his trunk tip on it, the acidic trunk mucus displaces **19** from the albumin.[76,140]

Slow release does not necessarily entail lower initial emission rates that reduce detectability. For example, fresh mouse urine contains signal volatiles that are not bound to MUPs and which generate the same gas phase concentrations as if MUPs were not present,[123] and which repel male mice.[121] The presence of fixatives can decouple scent-mark lifetimes from the MWs of their active constituents, allowing marks with very volatile active components to have long lifetimes and disrupting another possible source of patterns in signal chemistry.

Persistent emissions of low MW compounds can be generated by their *in situ* production. The liberation of odorous, volatile sulfur compounds from odorless, non-volatile precursors is widespread.[141] Felinine in the urine of domestic cats (*Felis catus*) is a precursor of 3-mercapto-3-methylbutanol ("tomcat thiol").[142] In the urine of male maned wolves (*Chrysocyon brachyurus*), the concentrations of 2-methylbut-3-en-2-ol, 3-methyl-1-methylthiobut-2-ene, and six odorous pyrazines increase with time for at least three days.[143] In the urine of both Asian and African elephant bulls, the concentrations of volatile ketones, alcohols, and alkyl phenols increase with standing at room temperature,[144] and in the urine of female African elephants, α-farnesene peaks after three days at room temperature, and β-farnesene and *exo*- and *endo*-brevicomin increase for at least five days.[145]

Rather than being a property specifically of scent mark components, signalling persistence may be conferred by detector sensitivity because low LODs prolong the period over which the declining emissions from a scent mark are detectable. Sniffer dogs can detect cadaver odor on carpet 65 days after it was deposited,[146] a period that is similar to the longest lifetimes reported for mammalian scent marks.

Because animals can remember odors and the signals that they carry, the effects of the signal from a scent mark can persist for longer than the lifetime of the scent mark itself. Mammals remember odors for periods that are well into the upper range of the known lifetimes of scent marks. Asian elephants remember odors for 16 weeks,[147] mice for 32 days,[148] and female genets (*Genetta genetta*) for nine weeks.[149] A special role for memory is supported by the presence in mammalian signals of two compounds that act as mnemonic triggers; the rabbit mammary pheromone **1** is a mnemonic trigger for the mother's odor,[150] and darcin in male mouse urine triggers single-trial learning of the site of the urine and its odor.[151] Remembrance of darcin lasts for at least 14 days, which is much longer than the volatile emissions from the scent mark.[151]

In summary, the range of potential signalling compounds that can operate under any given set of conditions is extended by *in situ* production, adsorption and absorption by fixatives, the formation of protected complexes, high detector sensitivity, and persistent effects on receivers, all of which free signal compounds from the need to be robustly stable to environmental conditions.

3.4 Costs

The costs of chemical signalling arise from fuelling the biosynthesis of signal compounds and their fixatives, and their release into the environment. Even the production of odors by commensal microbes requires hosts to provide substrates. If there are significant differences in the costs of biosynthesizing different compounds, selective pressures to generate signals from compounds that are metabolically inexpensive would generate patterns in signal chemistry.

Using metabolic by-products as signals imposes limited or no production costs. Male mice use both by-products and "purpose-built" signals to attract females; **18** is a microbially transformed by-product[50] and MUPs are synthesized *de novo*.[152] Male mice that scent mark more grow more slowly,[153] and hungry mice produce less MUP,[154] but the metabolic costs of MUPs and **18** have not been measured. We know of no reports on the differential costs of production of different chemical signals.

The MUPs and aphrodisin have a narrow range of MWs close to the lower end of the MW range for lipocalins, which as a class are small proteins.[155] The odor-binding albumin in the trunk mucus of Asian elephant bulls has a similar MW. All of these small proteins bind a single molecule of ligand, while the 66 kDa albumin in the urine of female Asian elephants, binds four molecules of the estrus pheromone **19**, at a ratio of 16.6 kDa of protein per molecule of ligand. This 16–19 kDa range might be the smallest protein motif that can form a lipophilic calyx that is both stable to environmental perturbations and responsive to functional manipulations, such as the change in pH when an Asian elephant bull's trunk mucus mixes with a cow's urine (see Section 3.2.2). The upper limit of the MW range might be the result of selection for low production cost; the smaller the protein, the lower its metabolic cost per unit of bound ligand.

3.5 Information content

3.5.1 Chemical diversity and signal diversity. Complex sociality based on individual identities requires a wide variety of social signals, and some authors have argued that coding a sufficient number of signals is possible only with multiple components and/or components of high MW.[3,14,99,156] These arguments seriously underestimate chemical diversity. Even the most conservative of *a priori* calculations of the number of possible natural organic compounds yield numbers in the tens

of millions, and the universe of small molecules (<500 Da) alone contains 10^{60} possible structures.[157] There is, in principle, a unique chemical compound for every biological message, hence signals are not constrained by chemistry to have more than one component, or components of a particular type.

The need for there to be a biosynthetic pathway leading to a signal or its precursor confines signal composition to a subset of the chemically possible structures, but substantial chemical diversity is still generated by the multiple pathways in amniote metabolism. For example, human breath[158] and urine[159] contain at least 2000 volatiles and more than 3100 small molecules, respectively, and the urine of inbred mice contains at least 3000 volatiles.[160] The urinary peptidome is similarly rich.[161,162]

Contra Charpentier *et al.*,[163] signal components do not have to be animal metabolites. Commensal microorganisms in scent glands and sacs, the surface of the skin, the gut, and the urinogenital tract generate volatiles that could serve as chemical signals (reviewed in Ezenwa and Williams 2014 (ref. 164)). **18**, a male mouse pheromone, is produced by microbial transformation of choline in the gut.[50] The microbes need not be animal commensals; **18**, ammonia, and species-typical sulfur volatiles are released from cat urine by soil microbes.[165]

This rich biochemical repertoire and an olfactory system with a broad scope and very narrow selectivity allow amniotes to generate robust redundancy in chemical signals by incorporating multiple compounds. In mice, five out of six of the male urine components that accelerate puberty in females are active alone.[166] The combination of **20** with 2-*sec*-butyl dihydrothiazole, and MUP3 both induce aggression by territorial male mice against castrates.[167] Male mouse urine contains three volatiles that attract females when spiked separately into castrate urine; **18**, **23**, and *Z*-5-tetradecen-1-ol.[50,66,88] Any single MUP of the several that are absent from a male mouse's MUP signature is sufficient to stimulate him to countermark spiked urine.[84] Either **22** or **25** from boars induce lordosis in sows when presented alone.[168] The three signal compounds in the urine of male tree shrews each elicit over-marking when presented singly.[97,98]

Redundancy in signal chemistry, and signals having one or a few components whose ratios are not critical to message integrity[18] are in sharp contrast to the situation in insects, where multicomponent ratio signals are common.[14] Mammals have *ca.* ten times as many different olfactory receptors as insects,[14] and it is possible that the broader olfactory scope and narrow selectivity of mammals allow them to use a wide diversity of compounds as single-component or redundant signals, while insects with a narrower olfactory scope have to generate specificity by combining chemically similar compounds in specific ratios.

3.5.2 Index signals – potential templates for signal patterns. Index signals are mechanistically linked to metabolic processes or other features of the signaller,[169] and these links could generate patterns in signal chemistry. Their components may be either the obligate by-products of metabolism, such as excreted steroid conjugates or the products of catabolism, or chemicals that have other roles besides signalling.[14,170]

3.5.2.1 Metabolic and physiological indicators. There are differences between taxa in signal chemistry; volatile signalling compounds that contain nitrogen or sulfur have been found only in mammals, where they are more common among signals from carnivores and insectivores than in signals from herbivores. This may be linked to their different diets and digestive physiologies providing different substrates for signal biosynthesis.

Excreted steroids and steroid metabolites are mechanistically linked to physiology, especially to stress and reproductive status, and their concentrations in urine and faeces are routinely measured to monitor reproductive condition and stress,[171] but they have attracted remarkably little attention from semiochemists. Apart from the androstene pheromones in the saliva of pigs (Table 1), and very recent work on sulfated estrogen,[172] there have been so few demonstrations of a semiochemical function for steroids that Baum and Bakker[173] (p. 281) remark, "The possible role of sex steroids as pheromonal signalling molecules remains a matter of speculation, based on a minimal amount of hard data".

Corticosteroids in milk have primer effects on the development of sucklings,[23,24,40] but their status as pheromones has not been recognised. DeCatanzaro[174] presents evidence that 17β-estradiol is a multifunctional primer pheromone in female mice that acts directly on target tissues after being absorbed into the bloodstream through the oral or nasal mucosa. Nevertheless, it is not clear how the females absorb the minimum effective dose of 140 ng per day when the highest concentration in male mouse urine is 20 ng ml^{-1}.[175] Two steroids, 5α-androst-2-en-17-one and -17β-ol, are present (probably as sulfates) in the urine of female Asian elephants, and their concentrations change with ovarian activity and the reproductive cycle,[176] but whether they act as cues or signals has not been tested. Testosterone and dihydrotestosterone concentrations in the temporal gland secretion of Asian and African elephant bulls fluctuate in parallel with serum concentrations, and are elevated during musth, although in Asian bulls they are not signals.[128] Small peptides are similarly likely to be index signals, and have been shown to signal sexual immaturity in female mice, and sexual maturity in male mice.[68,177] Some MUPs may be index signals in male mice; their production depends on nutritional status, and some of them appear to be involved in metabolic regulation.[154]

Neurophysiological results support a signalling role for excreted steroids; 71% of female mouse VNO sensory neurons respond selectively to sulfated steroids,[110,178] compared to 0.3–0.7% of VNO sensory neurons that respond to each of six known pheromones.[53] Nodari *et al.*[179] found that a large majority of mouse VNO sensory neurons respond to sulfated steroids, but not to native steroids, and that at physiological concentrations individual sensors are specific or selective. The mouse V1rj2 and V1rj3 VNO sensory neurons respond selectively to 1,3,5(10)-estratrien-3,17β-diol disulfate and 1,3,5(10)-estratrien-3,17β-diol 17-sulfate, which are components of an incompletely characterized mixture that stimulates males to mount receptive females.[177]

3.5.2.2 Dual-trait pheromones. Some compounds that act as pheromones have other concurrent functions. In the crested auklet (*Aethia cristatella*), a colonial seabird, both sexes possess special wick feathers that emit a mixture of even-numbered C_6–C_{12} aldehydes, which repel mosquitoes and impair lice and ticks (reviewed in Weldon and Carroll 2007 (ref. 180)) and also attract

conspecifics.[181,182] The crested auklet's odor is hypothesized to function as a signal of mate quality related to ectoparasite repellence.[183]

Antimicrobial activity is widespread among vertebrate secretions. Waxes from birds' uropygial glands, for example, inhibit feather-degrading bacteria.[184] During the nesting season, these glands in the green woodhoopoe (*Phoeniculus purpureus*) and European hoopoe (*Upupa epops*) secrete odorous antimicrobial volatiles that also repel predators.[185] (*E*)-3-Tridecen-2-one, a major volatile component of the interdigital gland secretion of black tailed deer (*Odocoileus hemionus columbianus*) inhibits the growth of bacteria and fungi.[186] This compound and other components of mammalian odors that exhibit antimicrobial properties[187] are potentially available as honest cues or signals to prospective mates and other conspecifics.

The pheromones of squamates are hypothesized to have evolved from integumentary lipids that contribute to the transepidermal water barrier.[31,118] If these pheromones currently contribute to impeding desiccation, they may provide cues denoting this homeostatic imperative to prospective mates.

Index signal compounds that are mechanistically linked to physiology and metabolism are likely sources of patterns in signal chemistry, but their role has been neglected. Monitoring hormones and other compounds in feces and urine, and as metabolic and disease indicators in urine, reveals how they vary with metabolic state, stress, and other factors.[188] Many of the relevant compounds are commercially available, hence their bioassay for signal function would be straightforward.

3.5.3 Chemical mimicry. Some within-species signals are deceptive. The most striking examples of such deception entail sexual mimicry, where males mimic females, and *vice versa*.[189] Sexual mimicry does not itself prescribe broad patterns of signal design. However, mimetic signals are constrained to match the prevailing honest signal patterns at the species or population level.

Sexual mimicry occurs in red-sided garter snakes. After emerging from hibernation, most males pass through a phase in which they are attractive to other males.[190] These so called "she-males" are believed to achieve a mating advantage by distracting other males in the mating balls of courting males that accumulate around females. In addition, the mating balls that form around she-males also transfer heat to them and reduce their exposure to predators.[191] GC-MS analyses of skin surface extracts of snakes during the breeding season reveal that females contain predominantly unsaturated methyl ketones, males contain predominantly saturated methyl ketones, and she-males are intermediate, containing both saturated and unsaturated methyl ketones.[192]

4 Summary and conclusions

The signalling compounds of amniotes are components of complex mixtures with diverse molecular weights and functionalities. The properties of these mixtures and specific components within them free the signalling compounds from some of the constraints imposed by requirements for signals to be stable, species-specific, persistent *etc.* In particular, the stability and emission of pheromones carried by scent marks are modulated by interactions with the matrix and by *in situ* production.

There are a few small-scale patterns among amniote chemical signals. Signal compounds with high MWs and low vapor pressures, or that are bound to carrier proteins, are detected during direct contact with the source of the signal. Stable compounds containing aromatic rings are more common in signals of social dominance, including territoriality. Aldehyde signal compounds are emitted from the sender's body rather than from scent marks, perhaps because their susceptibility to degradation renders them too short-lived to persist in scent marks. Lipocalin pheromones have a limited range of MWs, possibly to reduce the metabolic costs of their biosynthesis. Species-specificity is conferred by multicomponent signals. If this scarcity of patterns in current data is due to small sample size, biased data that are not comparable, and uneven taxonomic representation, then a large body of comparable, taxonomically diverse, unbiased data will reveal patterns that are currently obscured. Alternatively, a larger and better set of data might confirm that patterns really are sparse and small scale because the design constraints that might have channelled signal chemistry into patterns have been relaxed by amniote behavior and biochemistry. Signal detection imposes no practical constraints on the structures of signalling molecules because amniote olfaction has such a high sensitivity, wide range and narrow resolution, and carrier molecules and special behaviors transfer odors from the environment to the olfactory epithelia. The diversity of metabolic pathways in amniotes and their microbial commensals produces a wide variety of signal compounds. Semiochemicals do not have to be the products of amniote metabolism, and there is sufficient chemical and metabolic diversity for signals to be coded by both single compounds and mixtures. Metabolic diversity enables the production of complex mixtures of matrix compounds that free signal components from the constraints that would otherwise impose patterns on signal chemistry.

If there are any hidden patterns in amniote signal chemistry, they will be revealed by analyzing odors and the signal compounds they contain using methods whose results are comparable. The range of chemistries represented by the known signals confirms the need for correspondingly diverse analytical methods. Finding patterns in signal chemistry will also require investigation of a far wider and more representative range of species, and a far more rigorous approach to the designation of compounds as signals than prevails at present.

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6 References

1 B. W. Ache and J. M. Young, *Neuron*, 2005, **48**, 417–430.
2 W. H. Bossert and E. O. Wilson, *J. Theor. Biol.*, 1963, **5**, 443–469.
3 A. C. Alberts, *Am. Nat.*, 1992, **139**, S62–S89.

4 R. T. Mason and M. R. Parker, *J. Comp. Physiol., A*, 2010, **196**, 729–749.
5 P. J. Weldon, B. Flachsbarth and S. Schulz, *Nat. Prod. Rep.*, 2008, **25**, 738–756.
6 S. P. Caro and J. Balthazart, *J. Comp. Physiol., A*, 2010, **196**, 751–766; S. P. Caro, J. Balthazart and F. Bonadonna, *Horm. Behav.*, 2015, **68**, 25–42.
7 S. Campagna, J. Mardon, A. Celerier and F. Bonadonna, *Chem. Senses*, 2012, **37**, 3–25.
8 C. Dulac and A. T. Torello, *Nat. Rev. Neurosci.*, 2003, **4**, 551–562; C.-Y. Su, K. Menuz and J. R. Carlson, *Cell*, 2009, **139**, 45–59; S. D. Liberles, *Annu. Rev. Physiol.*, 2014, **76**, 151–175.
9 A. Petrulis, *Horm. Behav.*, 2013, **63**, 723–741.
10 J. A. Gottfried, *Curr. Opin. Neurobiol.*, 2009, **19**, 422–429.
11 P. I. Rekwot, D. Ogwu, E. O. Oyedipe and V. O. Sekoni, *Anim. Reprod. Sci.*, 2001, **65**, 157–170.
12 P. A. Brennan and F. Zufall, *Nature*, 2006, **444**, 308–315.
13 T. D. Wyatt, *Pheromones and Animal Behaviour, Communication by Smell and Taste*, Cambridge University Press, Cambridge, 2003.
14 T. D. Wyatt, *Pheromones and Animal Behaviour, Chemical Signals and Signatures*, Cambridge University Press, Cambridge, 2014.
15 A. Petrulis, *Horm. Behav.*, 2015, **68**, 1–2.
16 F. Regnier, *Biol. Reprod.*, 1971, **4**, 309–326; T. C. Baker, *J. Chem. Ecol.*, 2014, **40**, 310.
17 J. A. Pickett, *J. Chem. Ecol.*, 2014, **40**, 319.
18 P. J. Apps, *Naturwissenschaften*, 2013, **100**, 487–506.
19 B. Schaal, *Vitam. Horm.*, 2010, **83**, 83–136; R. Corona and F. Lévy, *Horm. Behav.*, 2015, **68**, 77–90.
20 B. Schaal, G. Coureaud, D. Langlois, C. Giniès, E. Sémon and G. Perrier, *Nature*, 2003, **424**, 68–72.
21 I. Brouette-Lahlou, R. Amouroux, F. Chastrette, J. Cosnier, J. Stoffelsma and E. Vernet-Maury, *J. Chem. Ecol.*, 1991, **17**, 1343–1354.
22 O. P. Love, P. O. McGowan and M. J. Sheriff, *Funct. Ecol.*, 2012, **27**, 81–92.
23 A. Catalani, P. Casolini, S. Scaccianoce, F. R. Patacchioli, P. Spinozzi and L. Angelucci, *Neuroscience*, 2000, **100**, 319–325; A. Catalani, P. Casolini, G. Cigliana, S. Scaccianoce, C. Consoli, C. Cinque, A. R. Zuena and L. Angelucci, *Pharmacol., Biochem. Behav.*, 2002, **73**, 105–114.
24 S. Macrì, P. Pasquali, L. T. Bonsignore, S. Pieretti, F. Cirulli, F. Chiarotti and G. Laviola, *PLoS One*, 2007, **2**, e1015; S. Macrì, O. Granstrem, M. Shumilina, F. J. Gomes dos Santos, A. Berry, L. Saso and G. Laviola, *Horm. Behav.*, 2009, **56**, 391–398; S. Macrì, F. Zoratto and G. Laviola, *Neurosci. Biobehav. Rev.*, 2011, **35**, 1534–1543.
25 J. delBarco-Trillo and C. M. Drea, *Anim. Behav.*, 2014, **97**, 249–253; T. E. Smith, A. J. Tomlinson, J. A. Mlotkiewicz and D. H. Abbott, *Chem. Senses*, 2001, **26**, 449–458; E. A. Macdonald, E. Fernandez-Duque, S. Evans and L. R. Hagey, *Am. J. Primatol.*, 2008, **70**, 12–18.
26 C. M. Drea, *Horm. Behav.*, 2015, **68**, 117–133.
27 R. M. Crewe, B. V. Burger, M. Le Roux and Z. Katsir, *J. Chem. Ecol.*, 1979, **5**, 861–868.
28 R. P. Michael, D. Zumpe, M. Richter and R. W. Bonsall, *Horm. Behav.*, 1977, **9**, 296–308.
29 D. A. Goldfoot, *Am. Zool.*, 1981, **21**, 153–164.
30 R. T. Mason, H. M. Fales, T. H. Jones, L. K. Pannell, J. W. Chinn and D. Crews, *Science*, 1989, **245**, 290–293.
31 R. T. Mason, T. H. Jones, H. M. Fales, L. K. Pannell and D. Crews, *J. Chem. Ecol.*, 1990, **16**, 2353–2369.
32 M. P. LeMaster and R. T. Mason, *J. Chem. Ecol.*, 2002, **28**, 1269–1285; M. P. LeMaster and R. T. Mason, *J. Chem. Ecol.*, 2003, **29**, 1027–1043.
33 T. D. Wyatt, *J. Comp. Physiol., A*, 2010, **196**, 685–700.
34 P. Karlson and M. Lüscher, *Nature*, 1959, **183**, 55–56.
35 *J. Chem. Ecol.*, 2008, **34**, 984.
36 R Core Team, *R: A Language and Environment for Statistical Computing*, The R Foundation for Statistical Computing, Vienna, Austria, 2014, http://www.R-project.org/.
37 D. Bates, M. Maechler, B. Bolker and S. Walker, *lme4: Linear Mixed-effects Models Using Eigen and S4, R package version 1.0-6*, 2014, http://CRAN.Rproject.org/package=lme4.
38 C. Weihs, U. Ligges, K. Luebke and N. Raabe, in *Data Analysis and Decision Support*, ed. D. Baier, R. Decker and L. Schmidt-Thieme, Springer-Verlag, Berlin, 2005, pp. 335–343.
39 W. N. Venables and B. D. Ripley, *Modern Applied Statistics with S*, Springer, New York, 4th edn, 2002.
40 K. Hinde, A. L. Skibiel, A. B. Foster, L. Del Rosso, S. P. Mendoza and J. P. Capitanio, *Behav. Ecol.*, 2015, **26**, 269–281.
41 L. M. Jackson and J. D. Harder, *Biol. Reprod.*, 1996, **54**, 506–512.
42 L. Mmualefe and P. J. Apps, unpublished work.
43 W. E. Grus, P. Shi, Y.-P. Zhang and J. Zhang, *Proc. Natl. Acad. Sci. U. S. A.*, 2005, **102**, 5767–5772; M. B. Kambere and R. P. Lane, *BMC Neurosci.*, 2007, **8**(suppl 3), S2.
44 M. Nei, Y. Niimura and M. Nozawa, *Nat. Rev. Genet.*, 2008, **9**, 951–963.
45 J. M. Young, M. Kambere, B. J. Trask and R. P. Lane, *Genome Res.*, 2005, **15**, 231–240; J. M. Young and B. J. Trask, *Trends Genet.*, 2007, **23**, 212–215.
46 W. E. Grus and J. Zhang, *Mol. Biol. Evol.*, 2008, **25**, 1593–1601.
47 H. Ohara, M. Nikaido, A. Date-Ito, K. Mogi, H. Okamura, N. Okada, Y. Takeuchi, Y. Mori and K. Hagino-Yamagishi, *BMC Evol. Biol.*, 2009, **9**, 233.
48 R. J. Beynon, J. L. Hurst, M. J. Turton, D. H. L. Robertson, S. D. Armstrong, S. A. Cheetham, D. Simpson, A. MacNicoll and R. E. Humphries, in *Chemical Signals in Vertebrates 11*, ed. J. L. Hurst, R. J. Beynon, S. C. Roberts and T. D. Wyatt, Spinger, New York, 2008, pp. 37–50.
49 A. J. Saviola, D. Chiszar, C. Busch and S. P. Mackessy, *BMC Biol.*, 2013, **11**, 20.
50 Q. Li, W. J. Korzan, D. M. Ferrero, R. B. Chang, D. S. Roy, M. Buchi, J. K. Lemon, A. W. Kaur, L. Stowers, M. Fendt and S. Liberles, *Curr. Biol.*, 2013, **23**, 11–20.
51 S. A. Roberts, D. M. Simpson, S. D. Armstrong, A. J. Davidson, D. H. Robertson, L. McLean, R. J. Beynon and J. L. Hurst, *BMC Biol.*, 2010, **8**, 75.

52 S. Takigami, T. Osada, J. Yoshida-Matsuoka, M. Matsuoka, Y. Mori and M. Ichikawa, *Neurosci. Lett.*, 1999, **272**, 115–118; L. B. Buck, *Cell*, 2000, **100**, 611–618; C. Dulac, *Curr. Opin. Neurobiol.*, 2000, **10**, 511–518; E. Pantages and C. Dulac, *Neuron*, 2000, **28**, 835–845.

53 M. Sam, S. Vora, B. Malnic, W. Ma, M. V. Novotny and L. B. Buck, *Nature*, 2001, **412**, 142.

54 M. Halpern and N. Frumin, *Physiol. Behav.*, 1979, **22**, 1183–1189; L. R. Miller and W. H. N. Gutzke, *Anim. Behav.*, 1999, **58**, 53–57; K. Trinh and D. R. Storm, *Nat. Neurosci.*, 2003, **6**, 519–525.

55 K. M. Dorries, E. Adkins-Regan and B. P. Halpern, *Brain Behav. Evol.*, 1997, **49**, 53–62; K. N. Baxi, K. M. Dorries and H. L. Eisthen, *Trends Neurosci.*, 2006, **29**, 1–7; W. T. Swaney and E. B. Keverne, *Behav. Brain Res.*, 2009, **200**, 239–247; M. Keller and F. Lévy, *Front. Neuroanat.*, 2012, **6**, 39; M. J. Baum and J. A. Cherry, *Horm. Behav.*, 2015, **68**, 53–64.

56 P. J. Weldon, R. Ortiz and T. R. Sharp, in *Biology of the Pitvipers*, ed. J. A. Campbell and E. D. Brodie Jr, Selva, Tyler, Texas, 1992, pp. 309–319; H. S. Fitch, G. R. Pisani, H. W. Greene, A. F. Echelle and M. Zerwekh, *Journal of Kansas Herpetology*, 2004, **11**, 18–24.

57 R. Shine and R. T. Mason, *Biol. Lett.*, 2012, **8**, 183–185.

58 D. G. Moulton and D. A. Marshall, *J. Comp. Physiol.*, 1976, **110**, 287–306.

59 L. Larsson and M. Laska, *Neurosci. Res.*, 2011, **71**, 355–360.

60 R. J. O'Connell, A. G. Singer, C. Pfaffmann and W. C. Agosta, *J. Chem. Ecol.*, 1979, **5**, 575–585.

61 A. Petrulis and R. E. Johnston, *Physiol. Behav.*, 1995, **57**, 779–784.

62 K. M. Dorries, E. Adkins-Regan and B. P. Halpern, *Physiol. Behav.*, 1995, **57**, 255–259.

63 D. Y. Lin, S.-Z. Zhang, E. Block and L. C. Katz, *Nature*, 2005, **434**, 470–477.

64 S. Can Güven and M. Laska, *PLoS One*, 2012, **7**, e34301.

65 X. Duan, E. Block, Z. Li, T. Connelly, J. Zhang, Z. Huang, X. Su, Y. Pan, L. Wu, Q. Chi, S. Thomas, S. Zhang, M. Ma, H. Matsunami, G.-Q. Chen and H. Zhuang, *Proc. Natl. Acad. Sci. U. S. A.*, 2012, **109**, 3492–3497.

66 E. Block and H. Zhuang, in *Biochalcogen Chemistry: The Biological Chemistry of Sulfur, Selenium, and Tellurium*, ed. C. A. Bayse and J. L. Brumaghim, ACS Symposium Series, Am. Chem. Soc., Washington, D.C., 2013, pp. 1–14.

67 J. He, L. Ma, S. Kim, J. Schwartz, M. Santilli, C. Wood, M. H. Durnin and C. R. Yu, *J. Neurosci.*, 2010, **30**, 7473–7483.

68 D. M. Ferrero, L. M. Moeller, T. Osakada, N. Horio, Q. Li, D. S. Roy, A. Cichy, M. Spehr, K. Touhara and S. D. Liberles, *Nature*, 2013, **502**, 368–371.

69 L. E. L. Rasmussen, T. D. Lee, A. Zhang, W. L. Roelofs and G. D. Daves, *Chem. Senses*, 1997, **22**, 417–437.

70 M. Dehnhard, H. Rohrmann and J. Kauffold, in *Chemical Signals in Vertebrates 12*, ed. M. L. East and M. Dehnhard, Springer, New York, 2013, pp. 381–390.

71 W. K. Whitten, M. C. Wilson, S. R. Wilson, J. W. Jorgenson, M. Novotny and M. Carmack, *J. Chem. Ecol.*, 1980, **6**, 49–55.

72 B. L. Hart and M. G. Leedy, *Horm. Behav.*, 1987, **21**, 44–52.

73 D. Müller-Schwarze, *Appl. Anim. Behav. Sci.*, 1991, **29**, 389–402.

74 K. B. Døving and D. Trotier, *J. Exp. Biol.*, 1998, **201**, 2913–2925.

75 G.-Z. Huang, J.-J. Zhang, D. Wang, R. T. Mason and M. Halpern, *Chem. Senses*, 2006, **31**, 521–529.

76 L. E. L. Rasmussen, J. Lazar and D. R. Greenwood, *Biochem. Soc. Trans.*, 2003, **31**, 137–141.

77 I. Brouette–Lahlou, F. Godinot and E. Vernet–Maury, *Physiol. Behav.*, 1999, **66**, 427–436.

78 M. Spehr, K. R. Kelliher, X. H. Li, T. Boehm, T. Leinders-Zufall and F. Zufall, *J. Neurosci.*, 2006, **26**, 1961–1970.

79 J. Vessman, R. I. Stefan, J. F. van Staden, K. Danzer, W. Lindner, D. T. Burns, A. Fajgelj and H. Müller, *Pure Appl. Chem.*, 2001, **73**, 1381–1386.

80 D. Rokni, V. Hemmelder, V. Kapoor and V. N. Murthy, *Nat. Neurosci.*, 2014, **17**, 1225–1232.

81 C. Bushdid, M. O. Magnasco, L. B. Vosshall and A. Keller, *Science*, 2014, **343**, 1370–1372; M. Meister, arXiv 1411.0165v1 [q-bio.NC], 2014.

82 S. K. Wasser, H. Smith, L. Madden, N. Marks and C. Vynne, *J. Wildl. Manage.*, 2009, **73**, 1233–1240; G. K. Beauchamp, K. Yamazaki, C. J. Wysocki, B. M. Slotnick, L. Thomas and E. A. Boyse, *Proc. Natl. Acad. Sci. U. S. A.*, 1985, **82**, 4186–4188; M. D. Thom and J. L. Hurst, *Ann. Zool. Fenn.*, 2004, **41**, 765–787.

83 T. Leinders-Zufall, P. Brennan, P. Widmayer, P. Chandramani, A. Maul-Pavicic, M. Jäger, X.-H. Li, H. Breer, F. Zufall and T. Boehm, *Science*, 2004, **306**, 1033–1037.

84 A. W. Kaur, T. Ackels, T.-H. Kuo, A. Cichy, S. Dey, C. Hays, M. Kateri, D. W. Logan, T. F. Marton, M. Spehr and L. Stowers, *Cell*, 2014, **157**, 676–688.

85 S. Yoshinaga, T. Sato, M. Hirakane, K. Esaki, T. Hamaguchi, S. Haga-Yamanaka, M. Tsunoda, H. Kimoto, I. Shimada, K. Touhara and H. Terasawa, *J. Biol. Chem.*, 2013, **288**, 16064–16072.

86 B. Malnic, J. Hirono, T. Sato and L. B. Buck, *Cell*, 1999, **96**, 713–723.

87 S. D. Munger, T. Leinders-Zufall, L. M. McDougall, R. E. Cockerham, A. Schmid, P. Wandernoth, G. Wennemuth, M. Biel, F. Zufall and K. R. Kelliher, *Curr. Biol.*, 2010, **20**, 1438–1444.

88 K. Yoshikawa, H. Nakagawa, N. Mori, H. Watanabe and K. Touhara, *Nat. Chem. Biol.*, 2013, **9**, 160–162.

89 C. Smadja and R. K. Butlin, *Heredity*, 2009, **102**, 77–97.

90 P. Apps, L. Mmualefe and J. W. McNutt, in *Chemical Signals in Vertebrates 12*, ed. M. L. East and M. Dehnhard, Springer, Berlin, 2013, pp. 417–432.

91 R. C. Karn, *Biochem. Genet.*, 1998, **36**, 105–117.

92 M. Laukaitis, E. S. Critser and R. C. Karn, *Evolution*, 1997, **51**, 2000–2005.

93 E. J. Uhrig, M. P. LeMaster and R. T. Mason, *Biochem. Syst. Ecol.*, 2014, **53**, 51–58.

94 M. Novotny, B. Jemiolo, S. Harvey, D. Wiesler and A. Marchlewska-Koj, *Science*, 1986, **231**, 722–725;

B. Jemiolo and M. Novotny, *Physiol. Behav.*, 1994, **55**, 519–522.
95 J.-X. Zhang, L. Sun, J.-H. Zhang and Z.-Y. Feng, *Chem. Senses*, 2008, **33**, 611–621.
96 X. Y.-H. Zhang and J.-X. Zhang, *Chem. Senses*, 2011, **36**, 125–135.
97 D. Müller-Schwarze and P. W. Houlihan, *J. Chem. Ecol.*, 1991, **17**, 715–734.
98 F. von Stralendorff, *Behav. Ecol. Sociobiol.*, 1982, **11**, 101–107.
99 F. von Stralendorff, *J. Chem. Ecol.*, 1987, **13**, 655–679.
100 P. López and J. Martín, *Chem. Senses*, 2009, **34**, 479–484.
101 B. G. Galef Jr, J. R. Mason, G. Preti and N. J. Bean, *Physiol. Behav.*, 1988, **42**, 119–124.
102 V. Būda, R. Mozūraitis, J. Kutra and A.-K. Borg-Karlson, *J. Chem. Ecol.*, 2012, **38**, 811–813.
103 B. L. Nielsen, N. Jerôme, A. Saint-Albin, C. Thonat, C. Briant, F. Boué, O. Rampin and Y. Maurin, *Behav. Brain Res.*, 2011, **225**, 584–589.
104 R. Over, J. Cohen-Tannoudji, M. Dehnhard, R. Claus and J. P. Signoret, *Physiol. Behav.*, 1990, **48**, 665–668.
105 T. Ichimaru, K. Mogi, S. Ohkura, Y. Mori and H. Okamura, *Anim. Reprod. Sci.*, 2008, **106**, 361–368.
106 P. J. Apps, D. Gordon, H. W. Viljoen and V. Pretorius, *J. Chem. Ecol.*, 1990, **16**, 2667–2676.
107 Y. Niimura, A. Matsui and K. Touhara, *Genome Res.*, 2014, **24**, 1485–1496.
108 T. Olender, T. Fuchs, C. Linhart, R. Shamir, M. Adams, F. Kalush, M. Khen and D. Lancet, *Genomics*, 2004, **83**, 361–372; A. Keller, *BMC Biol.*, 2012, **10**, 75.
109 X. Zhang, X. Zhang and S. Firestein, *Genomics*, 2007, **89**, 441–450.
110 Y. Isogai, S. Si, L. Pont-Lezica, T. Tan, V. Kapoor, V. N. Murthy and C. Dulac, *Nature*, 2011, **478**, 241–245.
111 M. Meredith and J. M. Westberry, *J. Neurosci.*, 2004, **24**, 5719–5725; M. Meredith, C. Samuelsen, C. Blake and J. Westberry, in *Chemical Signals in Vertebrates 11*, ed. J. L. Hurst, R. J. Beynon, S. C. Roberts and T. D. Wyatt, Spinger, New York, 2008, pp. 367–378.
112 J. L. Hurst and R. J. Beynon, *BioEssays*, 2004, **26**, 1288–1298.
113 F. S. Guthery and G. E. Mecozzi, *J. Wildl. Manage.*, 2008, **72**, 1175–1180; A. Gsell, J. Innes, P. de Monchy and D. Brunton, *Wildl. Res.*, 2010, **37**, 39–46; S. E. Reed, A. L. Bidlack, A. Hurt and W. M. Getz, *J. Wildl. Manage.*, 2011, **75**, 243–251.
114 E. G. Hammond, C. Fedler and G. Junk, *Trans. ASABE*, 1979, **22**, 1186–1189; L. Cai, J. A. Koziel, Y.-C. Lo and S. J. Hoff, *J. Chromatogr. A*, 2006, **1102**, 60–72.
115 G. S. Settles, D. A. Kester and L. J. Dodson-Dreibelbis, in *Sensors and Sensing in Biology and Engineering*, ed. F. G. Barth, J. A. C. Humphrey and T. W. Secomb, Springer, New York, 2002, pp. 323–335.
116 S. Gordon, L. A. Kiernan, M. J. Nieuwenhuijsen, A. D. Cook, R. D. Tee and A. J. N. Taylor, *Occup. Environ. Med.*, 1997, **54**, 135–140.
117 A. Schilling, *Symp. Zool. Soc. London*, 1980, **45**, 165–193.
118 B. M. Graves, D. Duvall, M. B. King, S. L. Lindstedt and W. A. Gern, in *Chemical Signals in Vertebrates 4, Ecology, Evolution, and Comparative Biology*, ed. D. Duvall, D. Müller-Schwarze and R. M. Silverstein, Plenum, New York, 1986, pp. 285–304.
119 R. E. Johnston and N. A. Lee, *Behav. Biol.*, 1976, **16**, 199–210; R. E. Johnston and T. Schmidt, *Behav. Neural Biol.*, 1979, **26**, 64–75.
120 M. L. Sipos, J. G. Nyby and M. F. Serran, *Physiol. Behav.*, 1993, **54**, 171–174.
121 J. L. Hurst, D. H. L. Robertson, U. Tolladay and R. J. Beynon, *Anim. Behav.*, 1998, **55**, 1289–1297.
122 S. C. Roberts, *Ethology*, 1998, **104**, 585–592.
123 J. Kwak, C. C. Grigsby, G. Preti, M. M. Rizki, K. Yamazaki and G. K. Beauchamp, *Physiol. Behav.*, 2013, **120**, 211–219.
124 M. L. Sipos, L. Alterman, B. Perry, J. G. Nyby and J. G. Vandenburgh, *Anim. Behav.*, 1995, **50**, 113–120.
125 O. A. E. Rasa, *Adv. Study Behav.*, 1987, **17**, 121–163.
126 J. L. Wellington, G. K. Beauchamp and A. B. Smith III, *Behav. Neural Biol.*, 1981, **32**, 364–375.
127 F. E. Regnier and M. Goodwin, in *Chemical Signals in Vertebrates*, ed. D. Müller-Schwarze and M. M. Mozell, Springer, New York, 1977, pp. 155–133.
128 L. E. L. Rasmussen and V. Krishnamurthy, *Zoo Biol.*, 2000, **19**, 405–423.
129 A. M. Belcher, A. B. Smith III, P. C. Jurs, B. Lavine and G. Epple, *J. Chem. Ecol.*, 1986, **12**, 513–531.
130 L. Briand, J.-C. Huet, V. Perez, G. Lenoir, C. Nespoulous, Y. Boucher, D. Trotier and J.-C. Pernollet, *FEBS Lett.*, 2000, **476**, 179–185.
131 J. Kwak, C. C. Grigsby, M. M. Rizki, G. Preti, M. Köksal, J. Josue, K. Yamazaki and G. K. Beauchamp, *Physiol. Behav.*, 2012, **107**, 112–120.
132 C. J. Austin, L. Emberson and P. Nicholls, *Eur. J. Biochem.*, 2004, **271**, 2593–2606.
133 W. D. Booth, *J. Reprod. Infertil.*, 1987, **81**, 427–431.
134 R. A. Hayes, B. J. Richardson and S. G. Wyllie, *J. Chem. Ecol.*, 2003, **29**, 1051–1064.
135 O. Redlich, C. M. Gable, A. K. Dunlop and R. W. Millar, *J. Am. Chem. Soc.*, 1950, **72**, 4153–4160; H. Schlenk and R. T. Holman, *Science*, 1950, **112**, 19–20; J. L. Iverson, J. Eisner and D. Firestone, *J. Am. Oil Chem. Soc.*, 1965, **42**, 1063–1068.
136 G. K. Beauchamp and J. Berüter, *Behav. Biol.*, 1973, **9**, 43–47.
137 A. Cavaggioni, C. Mucignat-Caretta, M. Redaelli and G. Zagotto, *Rapid Commun. Mass Spectrom.*, 2006, **20**, 3741–3746.
138 M. M. Phelan, L. McLean, S. D. Armstrong, J. L. Hurst, R. J. Beynon and L.-Y. Lian, *PLoS One*, 2014, **9**, e108415.
139 D. D. Thiessen, A. Clancy and M. Goodwin, *J. Chem. Ecol.*, 1976, **2**, 231–238.
140 J. Lazar, L. E. L. Rasmussen, D. R. Greenwood, I.-S. Bang and G. D. Prestwich, *Chem. Biol.*, 2004, **11**, 1093–1100.
141 C. Starkenmann, M. Troccaz and K. Howell, *Flavour Fragrance J.*, 2008, **23**, 369–381.
142 M. Miyazaki, T. Yamashita, Y. Suzuki, Y. Saito, S. Soeta, H. Taira and A. Suzuki, *Chem. Biol.*, 2006, **13**, 1071–1079; S. M. Rutherford, T. M. Kitson, A. D. Woolhouse,

M. C. McGrath and W. H. Hendricks, *Amino Acids*, 2007, **32**, 235–242.
143 T. E. Goodwin, N. Songsasen, L. J. Broederdorf, B. A. Burkert, C. J. Chen, S. R. Jackson, K. B. Keplinger, M. E. Rountree, Z. J. Waldrip, M. E. Weddell, L. P. Desrochers, W. K. Baker Jr and J. P. F. G. Helsper, in *Chemical Signals in Vertebrates 12*, ed. M. L. East and M. Dehnhard, Springer, Berlin, 2013, pp. 171–184.
144 T. E. Goodwin, L. J. Broederdorf, B. A. Burkert, H. H. Hirwa, D. B. Mark, Z. J. Waldrip, R. A. Kopper, M. V. Sutherland, E. W. Freeman, J. A. Hollister-Smith and B. A. Schulte, *J. Chem. Ecol.*, 2012, **38**, 81–87.
145 T. E. Goodwin, M. S. Eggert, S. J. House, M. E. Weddell, B. A. Schulte and L. E. L. Rasmussen, *J. Chem. Ecol.*, 2006, **32**, 1849–1853.
146 L. Oesterhelweg, S. Kröber, K. Rottmann, J. Willhöft, C. Braun, N. Thies, K. Püschel, J. Silkenath and A. Gehl, *Forensic Sci. Int.*, 2008, **174**, 35–39.
147 J. Arvidsson, M. Amundin and M. Laska, *Physiol. Behav.*, 2012, **105**, 809–814.
148 N. Bodyak and B. Slotnick, *Chem. Senses*, 1999, **24**, 637–645.
149 J. J. Roeder, *Z. Tierpsychol.*, 1983, **61**, 311–314.
150 B. Patris, B. Perrier, B. Schaal and G. Coureaud, *Anim. Behav.*, 2008, **76**, 305–314.
151 S. A. Roberts, A. J. Davidson, L. McLean, R. J. Beynon and J. L. Hurst, *Science*, 2012, **338**, 1462–1465.
152 S. D. Armstrong, D. H. Robertson, S. A. Cheetham, J. L. Hurst and R. J. Beynon, *Biochem. J.*, 2005, **391**, 343–350.
153 L. M. Gosling, S. C. Roberts, E. A. Thornton and M. J. Andrew, *Behav. Ecol. Sociobiol.*, 2000, **48**, 328–332.
154 K. Giller, P. Huebbe, F. Doering, K. Pallauf and G. Rimbach, *Proc. R. Soc. B*, 2013, **280**, 20130101.
155 D. R. Flower, *Biochem. J.*, 1996, **318**, 1–14.
156 R. E. Johnston, in *Chemical Signals in Vertebrates 10*, ed. R. T. Mason, M. P. LeMaster and D. Müller-Schwarze, Springer, New York, 2005, pp. 269–282.
157 P. Ertl, *J. Chem. Inf. Comput. Sci.*, 2003, **43**, 374–380; T. Fink, H. Bruggesser and J.-L. Reymond, *Angew. Chem., Int. Ed.*, 2005, **44**, 1504–1508; A. M. Virshup, J. Contreras-García, P. Wipf, W. Yang and D. N. Beratan, *J. Am. Chem. Soc.*, 2013, **135**, 7296–7303.
158 M. Phillips, R. N. Cataneo, A. Chaturvedi, P. D. Kaplan, M. Libardoni, M. Mundada, U. Patel and X. Zhang, *PLoS One*, 2013, **8**, e75274.
159 S. Bouatra, F. Aziat, R. Mandal, A. C. Guo, M. R. Wilson, C. Knox, T. C. Bjorndahl, R. Krishnamurthy, F. Saleem, P. Liu, Z. T. Dame, J. Poelzer, J. Huynh, F. S. Yallou, N. Psychogios, E. Dong, R. Bogumil, C. Roehring and D. S. Wishart, *PLoS One*, 2013, **8**, e73076.
160 M. L. Schaefer, K. Wongravee, M. E. Holmboe, N. M. Heinrich, S. J. Dixon, J. E. Zeskind, H. M. Kulaga, R. G. Brereton, R. R. Reed and J. M. Trevejo, *Chem. Senses*, 2010, **35**, 459–471.
161 P. R. Cutillas, *Methods Mol. Biol.*, 2010, **658**, 311–321.
162 P. Overath, T. Sturm and H. G. Rammensee, *Cell. Mol. Life Sci.*, 2014, **71**, 2429–2442.
163 M. J. E. Charpentier, N. Barthes, M. Proffit, J.-M. Bessière and C. Grison, *Funct. Ecol.*, 2012, **26**, 769–774.
164 V. O. Ezenwa and A. E. Williams, *BioEssays*, 2014, **36**, 847–854.
165 C. Starkenmann, Y. Niclass, I. Cayeux, R. Brauchli and A.-C. Gagnon, *Flavour Fragrance J.*, 2015, **30**, 91–100.
166 M. V. Novotny, W. Ma, D. Wiesler and L. Zídek, *Proc. R. Soc. B*, 1999, **266**, 2017–2022.
167 P. Chamero, T. F. Marton, D. W. Logan, K. Flanagan, J. R. Cruz, A. Saghatelian, B. F. Cravatt and L. Stowers, *Nature*, 2007, **450**, 899–902.
168 D. R. Melrose, H. C. B. Reed and R. L. S. Patterson, *Br. Vet. J.*, 1971, **127**, 497–501.
169 J. Maynard Smith and D. G. C. Harper, *Animal Signals*, Oxford University Press, Oxford, 2003.
170 B. A. Schulte, E. W. Freeman, T. E. Goodwin, J. Hollister-Smith and L. E. L. Rasmussen, *Appl. Anim. Behav. Sci.*, 2007, **102**, 344–363.
171 S. L. Monfort, in *Reproductive Science and Integrated Conservation*, ed. W. V. Holt, A. R. Pickard, J. C. Rodger and D. E. Wildt, Cambridge University Press, Cambridge, 2003, pp. 147–165; M. Heistermann, *Adv. Sci. Res.*, 2010, **5**, 1–9; D. C. Kersey and M. Dehnhard, *Gen. Comp. Endocrinol.*, 2014, **203**, 296–306.
172 S. Haga-Yamanaka, L. Ma, J. He, Q. Qiu, L. D. Lavis, L. L. Looger and C. R. Yu, *eLife*, 2014, **3**, e03025.
173 M. J. Baum and J. Bakker, *Front. Neuroendocrinol.*, 2013, **34**, 268–284.
174 D. DeCatanzaro, *Horm. Behav.*, 2015, **68**, 103–116.
175 D. DeCatanzaro, E. A. Beaton, A. Khan and E. Vella, *Reproduction*, 2006, **132**, 309–317.
176 M. Dehnhard, M. Heistermann, F. Göritz, R. Hermes, T. Hildebrandt and H. Haber, *Reproduction*, 2001, **121**, 475–484; M. Dehnhard, J.-M. Hatt, K. Eulenberger, A. Ochs and G. Strauss, *J. Steroid Biochem. Mol. Biol.*, 2003, **84**, 383–391.
177 S. Haga, T. Hattori, T. Sato, K. Sato, S. Matsuda, R. Kobayakawa, H. Sakano, Y. Yoshihara, T. Kikusui and K. Touhara, *Nature*, 2010, **466**, 118–122.
178 F. Celsi, A. D'Errico and A. Menini, *Chem. Senses*, 2012, **37**, 849–858.
179 F. Nodari, F.-F. Hsu, X. Fu, T. F. Holekamp, L.-F. Kao, J. Turk and T. E. Holy, *J. Neurosci.*, 2008, **28**, 6407–6418.
180 P. J. Weldon and J. F. Carroll, in *Insect Repellents: Principles, Methods, and Uses*, ed. M. Debboun, S. P. Frances and D. Strickman, CRC, Boca Raton, Florida, 2007, pp. 47–75.
181 J. C. Hagelin, I. L. Jones and L. E. L. Rasmussen, *Proc. R. Soc. London, Ser. B*, 2003, **270**, 1323–1329.
182 H. D. Douglas III, *Naturwissenschaften*, 2008, **95**, 45–53.
183 H. D. Douglas III, J. E. Co, T. H. Jones and W. E. Conner, *Naturwissenschaften*, 2001, **88**, 330–332.
184 J. Reneerkens, M. A. Versteegh, A. M. Schneider, T. Piersma and E. H. Burtt Jr, *Auk*, 2008, **125**, 285–290.
185 M. Martin-Vivaldi, A. Peña, J. M. Peralta-Sánchez, L. Sánchez, S. Ananou, M. Ruiz-Rodríguez and J. J. Soler, *Proc. R. Soc. B*, 2010, **277**, 123–130.

186 W. F. Wood, T. B. Shaffer and A. Kubo, *Experientia*, 1995, **51**, 368–369.
187 W. F. Wood, A. Kubo and T. B. Shaffer, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 1819–1820.
188 J. Kwak and G. Preti, in *Volatile Biomarkers Non-invasive Diagnosis in Physiology and Medicine*, ed. A. Amann and D. Smith, Elsevier, Oxford, 2013, pp. 395–404; B. L. Costello and N. M. Ratcliffe, in *Volatile Biomarkers Non-invasive Diagnosis in Physiology and Medicine*, ed. A. Amann and D. Smith, Elsevier, Oxford, 2013, pp. 405–462.
189 P. J. Weldon and G. M. Burghardt, *Z. Tierpsychol.*, 1984, **65**, 89–102.
190 R. T. Mason and D. Crews, *Nature*, 1985, **316**, 59–60; R. Shine, P. Harlow, M. P. LeMaster, I. T. Moore and R. T. Mason, *Anim. Behav.*, 2000, **59**, 349–359.
191 R. Shine, B. Phillips, H. Wayne, M. LeMaster and R. T. Mason, *Nature*, 2001, **414**, 267.
192 R. T. Mason, *Brain Behav. Evol.*, 1993, **41**, 261.
193 M. Novotny, S. Harvey, B. Jemiolo and J. Alberts, *Proc. Natl. Acad. Sci. U. S. A.*, 1985, **82**, 2059–2061.
194 M. V. Novotny, B. Jemiolo, D. Wiesler, W. Ma, S. Harvey, F. Xu, T. M. Xie and M. Carmack, *Chem. Biol.*, 1999, **6**, 377–383.
195 K. Ninomiya and T. Kimura, *Naturwissenschaften*, 1990, **77**, 586–588.
196 W. Ma, Z. Miao and M. V. Novotny, *Chem. Senses*, 1999, **24**, 289–293.
197 M. Novotny, S. Harvey and B. Jemiolo, *Experientia*, 1990, **46**, 109–113.
198 H. Arakawa, K. R. Kelliher, F. Zufall and S. D. Munger, *Chem. Senses*, 2013, **38**, 391–397.
199 H. Kimoto, K. Sato, F. Nodari, S. Haga, T. E. Holy and K. Touhara, *Curr. Biol.*, 2007, **17**, 1879–1884.
200 D. D. Thiessen, F. E. Regnier, M. Rice, M. Goodwin, N. Isaacks and N. Lawson, *Science*, 1974, **184**, 83–85.
201 A. G. Singer, F. Macrides, N. Clancy and C. Agosta, *J. Biol. Chem.*, 1986, **261**, 13323–13326.
202 L. Briand, D. Trotier and J.-C. Pernollet, *Peptides*, 2004, **25**, 1545–1552.
203 J. Cohen-Tannoudji, J. Einhorn and J. P. Signoret, *Physiol. Behav.*, 1994, **56**, 955–961.
204 K. Murata, S. Tamogami, M. Itou, Y. Ohkubo, Y. Wakabayashi, H. Watanabe, H. Okamura, Y. Takeeuchi and Y. Mori, *Curr. Biol.*, 2014, **24**, 681.
205 R. G. Brownlee, R. M. Silverstein, D. Müller-Schwarze and A. G. Singer, *Nature*, 1969, **221**, 284–285; D. Müller-Schwarze, R. M. Silverstein, C. Müller-Schwarze, A. G. Singer and N. J. Volkman, *J. Chem. Ecol.*, 1976, **2**, 389–398; D. Müller-Schwarze, U. Ravid, A. Claesson, A. G. Singer, R. M. Silverstein, C. Müller-Schwarze, N. J. Volkman, K. F. Zemanek and R. G. Butler, *J. Chem. Ecol.*, 1978, **4**, 247–256.
206 T. E. Perrin and L. E. L. Rasmussen, *J. Chem. Ecol.*, 1994, **20**, 2857–2866.
207 D. R. Greenwood, D. Comeskey, M. B. Hunt and L. E. L. Rasmussen, *Nature*, 2005, **438**, 1097–1098.
208 Z. Katsir and R. Crewe, *S. Afr. J. Zool.*, 1980, **15**, 249–254.
209 K. A. Flanagan, W. Webb and L. Stowers, *PLoS One*, 2011, **6**, e16660.
210 F. L. Rose, *Comp. Biochem. Physiol.*, 1970, **32**, 577–580.
211 E. R. Khannoon, A. El-Gendy and J. D. Hardege, *Chemoecology*, 2011, **21**, 143–149.
212 J.-X. Zhang, W. Wei, J.-H. Zhang and W.-H. Yang, *Chem. Senses*, 2010, **35**, 375–382.
213 M. Laska, M. Fendt, A. Wieser, T. Endres, L. T. Hernandez Salazar and R. Apfelbach, *Physiol. Behav.*, 2005, **84**, 211–215.
214 M. Laska, R. M. R. Bautista, D. Höfelmann, V. Sterlemann and L. T. Hernandez Salazar, *J. Exp. Biol.*, 2007, **210**, 4169–4178.
215 S. Kowalewsky, M. Dambach, B. Mauck and G. Dehnhardt, *Biol. Lett.*, 2006, **2**, 106–109.
216 P. K. Løtvedt, S. K. Murali, L. T. H. Salazar and M. Laska, *Pharmacol., Biochem. Behav.*, 2012, **101**, 450–457.
217 T. Leinders-Zufall, A. P. Lane, A. C. Puche, W. Ma, M. V. Novotny, M. T. Shipley and F. Zufall, *Nature*, 2000, **405**, 792–796.
218 C. Boschat, C. Pélofi, O. Randin, D. Roppolo, C. Lűscher, M. C. Broillet and I. Rodriguez, *Nat. Neurosci.*, 2002, **5**, 1261–1262.
219 S. D. Munger, T. Leinders-Zufall, L. M. McDougall, R. E. Cockerham, A. Schmid, P. Wandernoth, G. Wennemuth, M. Biel, F. Zufall and K. R. Kelliher, *Curr. Biol.*, 2010, **20**, 1438–1444.
220 J. L. Hurst, C. E. Payne, C. M. Nevison, A. D. Marie, R. E. Humphries, D. H. L. Robertson, A. Cavaggioni and R. J. Beynon, *Nature*, 2001, **414**, 631–634.